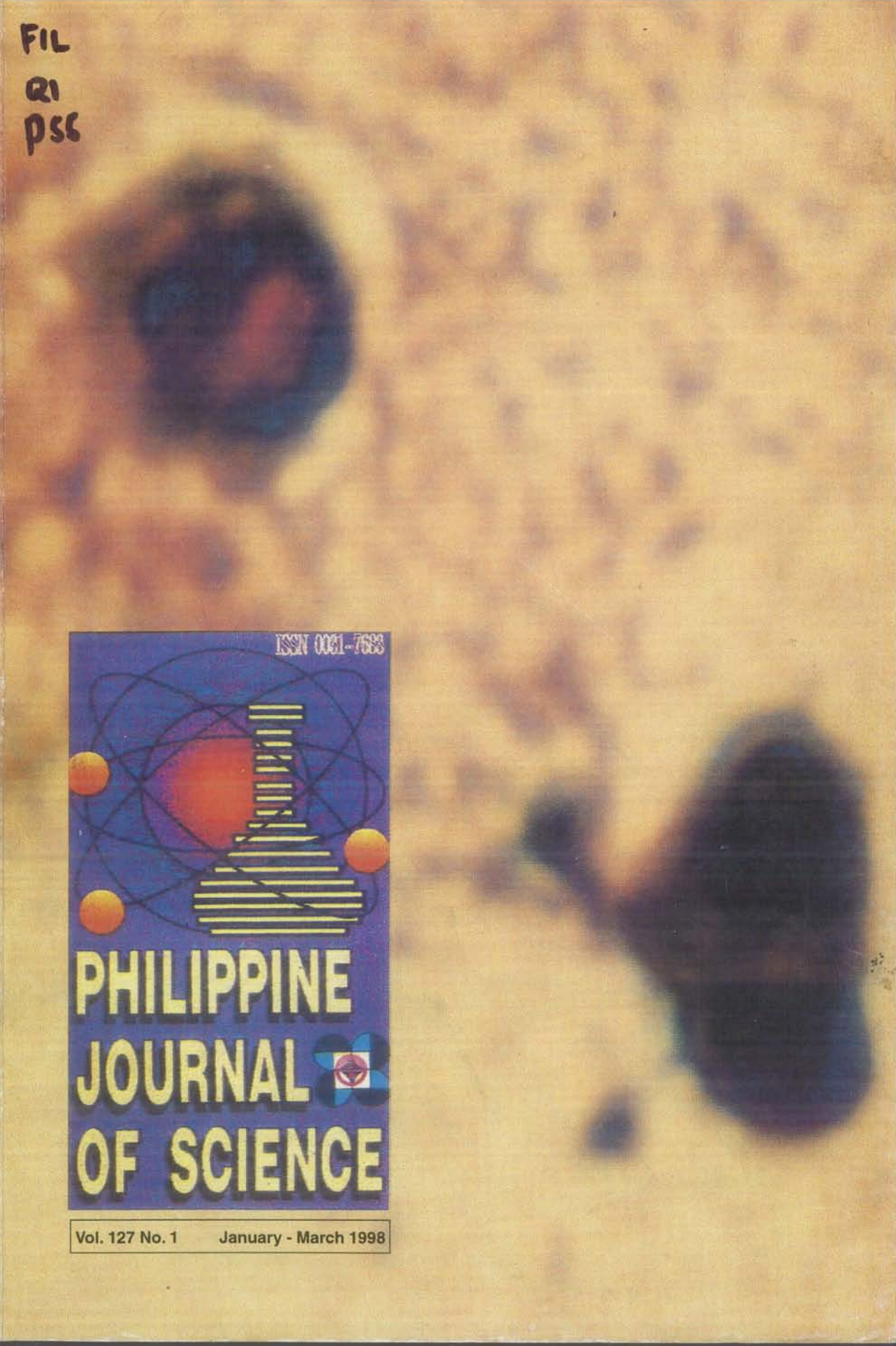

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OUR COVER

The cover, taken from the 2nd manuscript (Figure 7) published in this issue, is a photomicrographs of lymphocytes stimulated by phaseolus vulgaris lectin (PHA). A lymphocyte in the process of cell division is seen in the lower part (40x).

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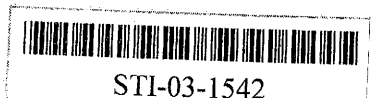

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Physico-Chemical and Phycological Investigation of the La Mesa Reservoir

JOSE SANTOS R. CARANDANG VI, JOHN MARY P. FORNILLOS RSM, NOEMI CELERINA F. DIMALIWAT and RAMONITO D. OROCEO
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ABSTRACT

Monthly investigations of the physico-chemical (surface temperature, Secchi disk transparency, pH, total alkalinity, conductivity, dissolved oxygen concentration, and nitrate and orthophosphate concentrations) and phycological (phytoplankton counts and gross primary productivity) characteristics of the La Mesa Reservoir in Novaliches, Quezon City were conducted from September 1993 to August 1994. The levels of most of the parameters tested do not indicate a pollution problem. However, the nutrient levels particularly the orthophosphate (0-0.85 mg/l) concentrations were at times, already high. Interestingly, the phytoplankton species count was low (27) despite an elevated total phytoplankton cell count (117-252.89 x 10^3 cells/l lake water). Chlorophytes were represented by 14 species while cyanobacteria had only 6 species identified. The observed increases in phytoplankton cell count and gross primary productivity, dissolved oxygen concentration and total alkalinity were correlated with one another. The increase occurred during the last half of the study (March - August 1994) when on the average, the surface temperature was higher by about 1°C than the first half.

INTRODUCTION

Of the 58 lakes in the Philippines, it is only Laguna de Bay which is monitored on a regular basis (EMB, 1990 & 1995). Analyses of water quality in the La Mesa Reservoir, which is located inside a rapidly growing metropolis, are mainly limited to those routinely done by the Metropolitan Waterworks and Sewerage System (MWSS) personnel as part of their standard operations and procedures. Short term and often unrelated student researches are also sporadically performed. In fact, the La Mesa Reservoir was not even included in the survey conducted by Darvain et al. (1984) on lakes and reservoir found in the island of Luzon,

Philippines. The results of the present study will be useful in the evaluation of the water quality of La Mesa Reservoir. The lake serves as the impoundment of raw water for the Balara Filtration facilities which in turn supplies a big part of Metro-Manila with potable water.

Area of Investigation

The La Mesa Reservoir is located in Quezon City, Philippines. The reservation has a total area of 27 km^2. About 3.5 km^2 is covered by the lake depending on the water elevation and the rest by a forested area. However, the vicinity of the reservation is already highly populated (refer to Fig. 1). The reservoir is man-made and was formed when the dam was built in 1929. The water inflow into the reservoir varies (1,200-1,600 x 10^6 l/d) depending on the needed raw water of the Metropolitan Waterworks and Sewerage System (MWSS) Balara Filtration Facilities. The two sampling stations which were about 500 m away from each other, were situated near the outlet towers of the dam.

Figure 1. A map showing the location of La Mesa Reservoir. The area covered by this map is bounded approximately by the coordinates - 14°40' South, 14°45' North, 121°03' West and 121°07' East. The map is drawn in the scale of 1:50,000.

MATERIALS AND METHODS

The data presented in this paper is from a larger study which aimed to compare the water quality of the reservoir with that of Laguna de Bay. Although the average depth of the reservoir have ranged from around 73 to 80 meters, sampling have been limited to daytime collections from surface waters only, mainly because of the large numbers of parameters to be tested. The samples (both water and phytoplankton) were collected monthly for six months at mid-morning. The collection months are numerically designated in the figures (Fig. 2-3) starting from September 1993 (the first collection month) up to August 1994 (the twelfth and last month). The reported data are the grand averages while the standard deviations are indicated by bars in the illustrations. The correlation between the parameters tested were also analyzed. The required R for significance (absolute value) at 0.05 level of confidence was 0.402 and 0.513 for the 0.01 level of confidence.

Physico-chemical parameters

The analyses of all physical parameters were done in situ. The surface temperature was determined by mercury thermometer. Transparency was measured using a Secchi disk. The analyses of certain chemical factors were also done in situ. Conductivity was determined by a YSL Model SCT (salinity and conductivity meter). Fixation of water samples in BOD bottles for dissolved oxygen analysis by Winkler Method and the dark and light bottles technique for analysis of primary productivity (Thomas, 1988) were done in the field while the titrations were performed in the laboratory. For total alkalinity and nutrient analyses, 2 liters of water sample were collected just below the water line from each sampling station. The total alkalinity was estimated using the titrimetric method (phenolphthalein and methyl orange method) as described by Umaly and Cuvin (1988). The nitrate content (cadmium reduction method) and the orthophosphate content (ascorbic acid method) of the collected water sample were determined using a spectrophotometer (Cole Parmer Spectronic 20) as described by the USEPA (1979).

Phytoplankton collection:

Phytoplankton were collected by the passing 25 liters of surface water (from up to 30 cm. deep) into a Wildco 48 C60 plankton net (80μm mesh). Phytoplankton were counted using the Sedgewick Rafter Method as described by Leopold (1988) and Schoen (1988). Identification were based on Prescott (1964), Shirota (1966), Pantastico (1977) and Umaly and Cuvin (1988). The Philippine National Museum and the Philippine Fisheries and Aquatic Resources Laboratory verified the species identifications. The number of species present from each site and the number of individuals counted from each species identified were recorded.

RESULTS

Physico-chemical data

Table 1 enumerates the results of the physico-chemical measurements conducted. The La Mesa Reservoir surface temperature did not vary much while the Secchi disk transparency averaged more than 1.5 m. The pH was from neutral to slightly alkaline. Similar to the trends in the dissolved oxygen concentration readings, the higher counts for alkalinity were also observed during the second half of the study. However, the nutrient content was high most of the time. On the other hand, the conductivity of La Mesa Reservoir was low.

Table 1. Summary of some physical and chemical characteristics of La Mesa Lake.

Parameters	Unit	Mean Values	Range	*DENR Standards
surface temperature	°C	28.7	27-32.8	max. rise of 3°C from ambient
transparency	m	1.74	0.87-2.25	–
pH		7.4	6.8-8.1	6.5–8.5
conductivity	μmhos/cm	300.4	137-2050	–
dissolved oxygen concentration	mg/l	11.9	4.3-19.4	≥ 5
total alkalinity	mg/l	35.2	11.5-71.5	–
nitrate concentration	mg/l	0.25	0-0.63	≤ 10
orthophosphate concentration	mg/l	0.3	0-0.85	≤ 0.1 (as total phosphate)

*These are standards for Public Water Supply Class II from the Water Quality Criteria for Conventional and other Pollutants Contributing to Aesthetics and Oxygen Demand for Fresh Waters (Reference: DENR Administrative Order #34)

Figure 2 compares the seasonal fluctuations of 4 parameters (dissolved oxygen content, phytoplankton cell count, total alkalinity and the gross primary productivity). Each parameter showed an increasing trend. The main increase for each of these factors started around March 1994 which corresponds to the start of the 'warmer months' when the photoperiod is also slightly longer.

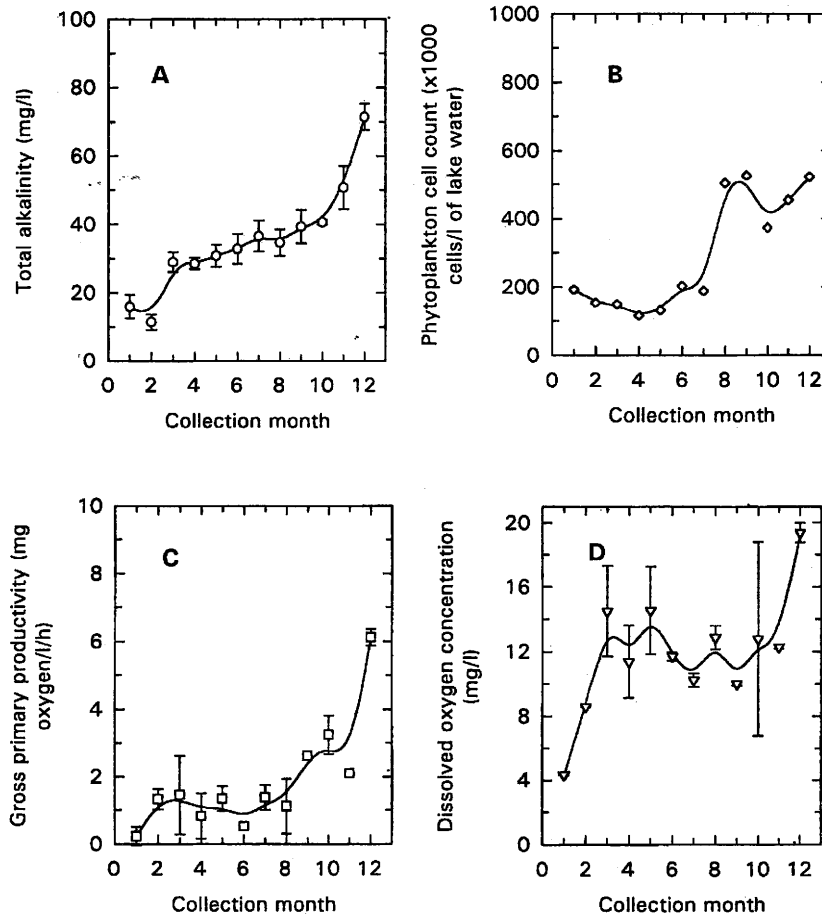

Figure 2. A comparison of the seasonal fluctuations of 4 monitored parameters (A. total alkalinity; B. phytoplankton cell count, C. gross primariy productivity and D. dissolved oxygen by division).

Phycological data

The total phytoplankton cell count (117.525.89 x10^3 cells/l of lake water; Fig. 2B) was high. However, Table 2 indicates a low species count (27) for La Mesa Lake. The collected phytoplankton belong to divisions Cyanophyta, Chlorophyta, Pyrrophyta and Bacillarophyta. Five species (*Anabaena sphaerica, Nostoc linckia, Ankistrodesmus convulatus, Treubaria appendiculata* and *Navicula gracilis*) were detected only starting March 1994 when a marked increases in cell counts were observed. More than half of the identified phytoplankton were green algae with only a few (6) cyanobacteria (Fig. 2).

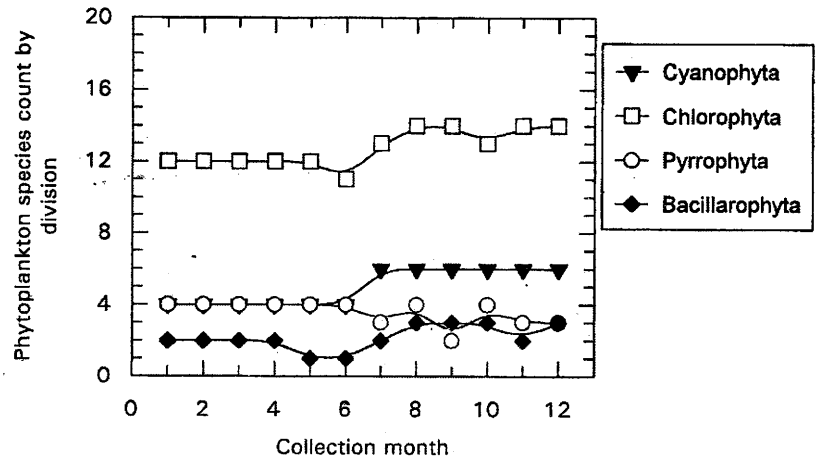

Figure 3. The La Mesa lake phytoplankton species count by division.

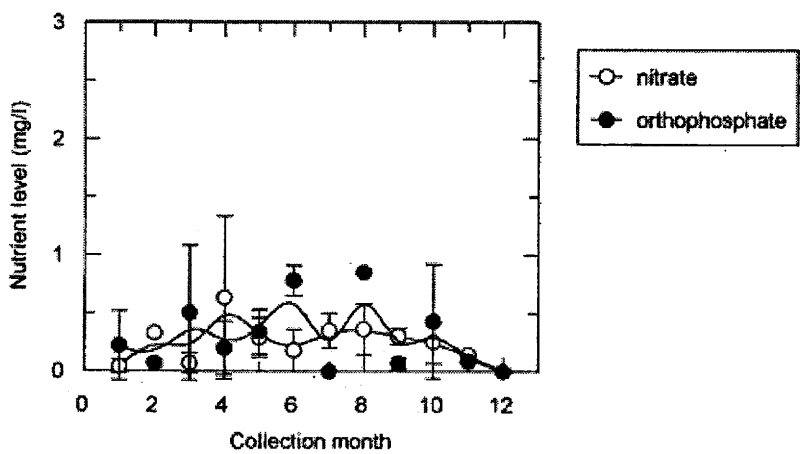

Figure 4. The La Mesa Reservoir nutrient levels.

Table 2. List of identified phytoplankton species.

Cyanophyta

1. *Anabaena sphaerica* Bornet et. Flahault
2. *Nostoc linckia* Vaucher
3. *Oscillatoria ornata* Kuetz.
4. *Oscillatoria princeps* Vaucher
5. *Spirulina major* Kuetz.
6. *Spirulina princepus* (W. et. G. S. West) G. S. West

Chlorophyta

7. *Ankistrodesmus convulatus* (Corda)
8. *Ankistrodesmus falcatus* (Corda) Ralfs
9. *Chlorella vulgaris* (Beij.)
10. *Microspora willeane* Wittr.
11. *Oedogonia crispum* (Hass.) Wittr.
12. *Oocystis Borgei* Snow
13. *Pediastrum clathratum* Lemm.
14. *Pediastrum simplex var duodenarium* (Bailey) Rab.
15. *Raphidonema nivale* Lag.
16. *Sorastrum spinolum* Ralfs
17. *Staurastrum rotula* Nordst.
18. *Treubaria appendiculata* Bernard
19. *Ulothrix cylindricum* Kuetz.
20. *Zoochlorella sp.*

Pyrrophyta

21. *Ceratium candelabrum* (Ehr.) Stein
22. *Ceratium extensum* Ehr.
23. *Ceratium furca* Ehr.
24. *Ceratium hirundinella* (O. F. M.) Schrank

Bacillarophyta

25. *Amphipleura pellucida* Kuetz.
26. *Diatomella sp.*
27. *Navicula gracilis* Ehr.

DISCUSSION

To facilitate the discussion, the data collected were compared with those reported from other lakes and reservoir. Although most of the lakes cited were from Luzon, their sizes range from the largest in Southeast Asia, Laguna de Bay with 90,000 ha., to Sampaloc Lake which has an area of only 102 ha. Data from volcanic lakes e.g. Taal Lake and Lake Lanao in Mindanao were also used for comparison. Likewise, information from other reservoir including Lake Caliraya, Pantabangan Dam and Ambuklao Dam were cited.

Physico-chemical data

The La Mesa Reservoir surface temperature readings did not vary much in relation with time, with slightly higher readings (around 1°C higher) during the second half of the study. It ranged from 27°C in February 1993 to 32.8°C in October 1993 with an average of 28.7°C. The temperature readings were similar to those reported for other Philippine lakes i.e. 25-31°C for Laguna de Bay at Mayondon Los Baños (Tamayo-Zafaralla et al., 1990) and 27-30°C for Taal Lake (Zafaralla et al., 1992). Similar to what Barril (1993) has described for Laguna de Bay, the temperature trends of La Mesa Reservoir is typical to tropical lakes where low temperatures (<30°C) generally occur from November to March and high temperature (>30°C) from April to October. The capacity of warm water to select organisms is notable. For example, cyanobacteria are most tolerant to elevated temperature and may even become dominant when temperatures are maintained higher than 32°C for any length of time. However, the surface temperature was most of the time, below 32°C.

Transparency is known to range from a few centimeters in very turbid conditions and even up to 40 m in very clear unproductive lakes found in high altitudes (Odum, 1971). A decrease in transparency results to a poor quality of light penetrating the water, which leads to reduced photosynthetic activity and thereby lowering primary productivity. Results indicate that La Mesa Reservoir is relatively clear (the average Secchi disk transparency was 1.74 m) with apparent color of light green. Interestingly, these characters are often observed in less productive lakes (Ruttner, 1963; Reid and Wood, 1976). Likewise, the La Mesa Reservoir transparency was comparable to other Philippine lakes and reservoir. Lake Caliraya had 0.2 m, 0.87 m for Lake Bustos, 1.1 m for Pantabangan Dam, 1.29 m for Lake Binga, 2.0 m for Ambuklao Dam (Darvain et al., 1984), and 0.1-1.4 m for the West Arm of Laguna de Bay (Barril, 1993).

On the average, the pH of La Mesa Reservoir is slightly alkaline (pH 7.4) and ranged from pH 6.8-8.1. These values were within the prescribed range for reservoirs (pH 6.5-8.5; Tab. 1) but were slightly lower than those reported for Laguna de Bay in 1993 by Barril (pH 7.8-9.7) and DENR-PRRP (pH 7.7-8.0 with a high of pH 10). The high pH readings in the latter were attributed to intense phytoplankton photosynthesis. However, the pH of La Mesa Reservoir was in the vicinity of those reported for other Philippine lakes and reservoir. In a comparative study of 25 Philippine lakes and reservoir by Darvain et al. (1984) the lowest pH readings reported were from Gunao Lake (pH 6.1-8.4) while the highest were from Sampaloc Lake. It must be mentioned here that a pH value beyond the range of 6.5-9.5 units may already affect the reproduction and growth of fish while levels below pH 4 or above pH 11 are already lethal (Boyd, 1982).

The total alkalinity of La Mesa Reservoir ranged from 11.5-71.5 mg/l with an average of 35.2 mg/l. These values were within the levels (<400 mg/l) expected from natural waters. Although natural water with a total alkalinity of

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40 mg/l or more are considered more productive than waters of lower alkalinity (Boyd, 1982), highly alkaline waters are usually distasteful and not used for domestic supply (Umaly and Cuvin, 1988).

The average conductivity of La Mesa Reservoir (300.4 µmhos/cm) was within the range reported by Tamayo-Zafaralla (1990) for Laguna de Bay at Mayondon, Los Baños (390-1050 µmhos/cm) but was lower than those reported for Taal Lake (1730-1780 µmhos/cm) by Darvain et al. (1984). Lakes with electrical conductivity ranging from 600-6000 µmhos/cm can be considered as already relatively saline. The conductivity readings of La Mesa Reservoir, thus confirms its freshwater status.

On the average, the dissolved oxygen content (11.9 mg/l) of La Mesa Reservoir was high (reaching a peak of 19.4 mg/l in August 1994), although a low reading of 4.3 mg/l (which is even lower than the value prescribed for reservoirs; see Tab 1) was recorded in September 1993. These values were slightly higher than those (6.7-11 mg/l) reported by Barril (1993) for Laguna de Bay. However, eutrophic lakes were also observed to exhibit extremely high DO levels (Jeffries and Mills, 1990 and Mason, 1991). Algal blooms for example may lead to supersaturation of dissolved oxygen during the day but their respiratory requirements in the evening may result in fish kills.

Mineral supply and phytoplankton productivity

The highest orthophosphate reading for La Mesa Reservoir was in April 1994 (0.85 mg/l) while the lowest (undetectable) was recorded in March 1994 (Fig 4). On the average, the La Mesa Reservoir orthophosphate levels apparently exceeded the Philippine standard for raw water source for domestic use (0.1 mg/l: Tab 1). It was also higher than the surface water quality objective of Alberta (Canada) for total phosphorous (0.15 mg/l) and the US marine aquatic maximum concentration for phosphorous (P_2O_5) of 5.0 mg/l (Makepeace et al., 1995). Other Philippine lakes, Laguna de Bay (0.092-0.11 mg/l; Barril, 1993) and Taal Lake (0.05-0.28 mg/l; Zafaralla et al., 1992) were reported to have phosphate values in the vicinity of the La Mesa Reservoir levels.

The total phosphorous (the sum of inorganic phosphate ions, inorganic polymers and organic phosphorous compounds) content of a water is a reasonable measure of its fertility and dissolved phosphates are generally present within the range of 0.1-1000 µg/l (Reynolds, 1984). A concentration of 1 µg/l suggests that the water is infertile while 1 mg/l or more suggests that it is fertile (Moss, 1988). Dissolved phosphates are mainly derived from weathering of phosphatic minerals but the high orthophosphate levels in La Mesa Reservoir, may be traced to contamination with domestic sewage as well as run-off fertilizers from agricultural activities in the surrounding areas of the lake. Domestic wastewater for example, contain up to 4 g/l orthophosphate (Henze, 1995). Likewise, urban stormwater that apparently also drain into the lake, contain up to 3.52 mg/l soluble phosphorous (Makepeace et al., 1995).

The La Mesa Reservoir nitrate concentration (0-0.63 mg/l: see Fig 4) was in the vicinity of those reported by Zafaralla et al. (1990) for Laguna de Bay at Mayondon, Los Baños (0-0.6 mg/l) and Tamayo-Zafaralla (1992) for Taal Lake (0.21-0.43 mg/l). Apparently, the nitrate level of La Mesa Reservoir was consistently lower than the Philippine criterion (10 mg/l nitrate: Tab 1) for sources of water supply. Furthermore, the La Mesa Reservoir nitrate level was within the Alberta (Canada) surface water quality objective of 1.0 mg/l for total nitrogen but was also lower than the drinking water quality guideline of the USA and WHO (44 mg/l nitrate) and EEC (50 mg/l nitrate) as described by Makepeace et al. (1995). However, the critical nitrogen level for freshwater lakes (0.3 mg/l; Barril, 1993) were at times apparently already exceeded in La Mesa Reservoir.

According to Reynolds (1984), waters receiving significant run-off from agricultural soils, ground water or treated sewage effluents may contain inorganic nitrate in the order of mg/l which otherwise would only be two orders of magnitude lower (i.e. 10-10000 µg/l). Even very dilute domestic wastewater contain 0.5 g/l nitrate-nitrogen (Henze, 1995) while stormwater contain up to 12 mg/l nitrate-nitrogen (Makepeace et al., 1995). Indeed, waters that are rich in nitrate-nitrogen could be considered as having been polluted (cultural eutrophication) for a long time but has undergone stabilization. Such waters are less hazardous to public health than those which are rich in organic nitrogen and ammonia. However, high levels of nitrate should already serve as a warning for potential algal bloom.

The nitrogen content of a water is usually higher than its phosphorous content because the former is more readily available than the latter. Consequently, more nitrogen is used by organisms in comparison with phosphorous. In order to produce 100 units of algae for example, 5 units of nitrogen and only 0.08 units of phosphorous (a ratio of 60N:1P) would be required (Machentun, 1969). However, the calculated N:P ratio (0.83) is even much lower than those reported from 2 eutrophicated ponds which is also located near an urban development (3.0-5.7 and 16.4-26.3; Wild et al., 1995). This may indicate that the level of phosphate input into La Mesa Reservoir was faster than the rate of incorporation, deposition or washing out. The observed elevated levels of orthophosphate is expected to enhance primary production which accompany algal blooms Mason (1991). However, the estimated gross primary productivity were not significantly correlated (-3.21) with the orthophosphate concentration. Even the correlation between the nitrate levels and the gross primary productivity were also insignificant (-1.91).

Phycological data

A low total of 27 phytoplankton species belonging to 4 divisions, 4 classes, 7 orders and 10 families were identified. Apparently, more species (15%) were represented from April to August 1994 resulting to cell counts which were more than double than those recorded for the months September 1993 to March 1994. Similar to Lake Lanao (Lewis, 1978) and Laguna de Bay

(URSI, 1989), Chlorophyta had the most number of species in La Mesa Reservoir. Although division cyanophyta only had 6 species in the present study, cyanobacteria had been reported to be the most diversified group in Laguna de Bay where a total of 33 phytoplankton species were identified (URSI, 1989). The four divisions represented in La Mesa Reservoir (Chlorophyta, Cyanophyta, Pyrrophyta and Bacillarophyta) were also recorded from Laguna de Bay at Mayondon, Los Baños (Tamayo-Zafaralla, 1990). Zafaralla et al., (1992) on the other hand, reported only three divisions (Chlorophyta, Cyanophyta and Bacillarophyta) from Taal Lake.

The low La Mesa Reservoir species count (with conspicuously few cyanobacteria and diatom species) could be due to the relatively large mesh size (80 μm) of the plankton net used. Smaller cells were thus, not collected. However, the species count may be affected also by the apparent lack of mixing (turbulence) in the lake attributed to it being a reservoir. Cyanobacteria which often have large cells with no floating devices (e.g. gas vesicles) together with centric diatoms that have heavy cells, require a degree of turbulence to maintain them in suspension (Moss, 1988). Likewise, high nitrate concentrations have been reported to inhibit the development of nitrogen fixing species (Leonardson and Bengtson, 1978). Furthermore, grazing zooplankton are known to be abundant during what is called the 'clear water phase' in most lakes (Sommer et al., 1986; Lampert and Sommer, 1993; Sommer, 1994). It is possible that the apparent clarity of La Mesa Reservoir allowed unabated zooplankton grazing which keep certain phytoplankton populations too low to be detected.

Interaction of factors

Most of the parameters tested (i.e. surface temperature, pH, total alkalinity and dissolved oxygen concentration) appear to be in the normal range described by Wandan and Zabik (1995). However, the nutrient concentrations were at times already high. The phytoplankton cell counts were also elevated (ranging from 117 x 10^3 cells/l in December 1993 to 525.89 x 10^3 cells/l in May 1994) and were much higher than the estimate (1-10 x 10^3 cells/l) of Sommer (1994) for plankton cell counts in natural waters. In temperate lakes, major changes in the seasonal succession of phytoplankton had been attributed (Wetzel; 1983; Reynolds, 1984; Moss, 1988) to the changes in the availability of nutrients (phosphorous, nitrogen and silica). However, the phytoplankton cell count in the present study was not correlated to either the nitrate concentration (-0.60) or the orthophosphate concentration (+0.070). There was also no correlation (+0.026) between cell count and surface temperature, although the increase in phytoplankton cell count occurred during the period when the surface temperatures were slightly higher by around 1°C.

What seems to be the case is that, an increase in alkalinity had lead to an increase in cell count (+0.463) and in gross primary productivity (+0.802). It is logical that the higher the phytoplankton cell count, the higher is then the gross primary productivity which was verified by an R value of (0.532). In

turn, an increase in gross primary productivity or increased photosynthetic rates lead to an increase in the dissolved oxygen content in water (+0.471). The interaction then goes in full circle since an increase in dissolved oxygen content lead to an increase in alkalinity (+0.661). As already stated, a heightened photosynthetic activity leads to more oxygen production but the same increased activity may exhaust the carbon dioxide supply forcing the system to use bicarbonate ions as carbon source. Hydroxyl ions may then be secreted to replace the bicarbonate ions utilized for photosynthesis while some of the freed carbon dioxide will precipitate as calcium carbonate leading to an increase in pH (Jeffries and Mills, 1990).

CONCLUSION

Most of the parameters tested do not indicate a pollution problem. However, the nutrient levels particularly the orthophosphate concentrations, were at times already high. Interestingly, the increase in cell count during the second half of the study was apparently not directly due to the high nutrient levels but as a response to other conditions of existence.

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Two Lectins from the Seeds of Lablab Bean (*Lablab purpureus* Linn cv. Highworth)

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ABSTRACT

Two mitogenic lectins were isolated from the seeds of lablab bean (Lablab purpureus Linn cv Highworth) by protein extraction using phosphate buffered saline (PBS) pH 7.2, ammonium sulfate fractionation and gel permeation chromatography using superfine Sephadex G-200.

Both lectins were non-blood type specific but blood group specific because they agglutinated untreated blood types A, B, O and AB but not trypsinized and untrypsinized blood from calf and goat. Hemagglutination by lectin 1 was inhibited by the addition of D-fructose, D-glucose, maltose, sucrose and methyl-α-D-mannopyranoside using blood type O and by D-glucose and D-mannose using blood type B. Lectin 2 was inhibited by D-fructose, D-mannose, D-glucose and methyl-α-D-mannopyranoside regardless of blood type. Among the haptenic sugars, strongest inhibition was elicited by methyl-α-D-mannopyranoside. Agglutination of blood types A and AB by lectin 1 was not inhibited by any of the sugars used.

Precipitation of lectins 1 and 2 occurred at pH 4-5 and 8-9 respectively, indicating that their isoelectric point lie in these ranges and that the former is a basic protein while the latter is acidic. Activity of both lectins were stable from pH 2-12. Extreme temperatures destroyed the activity of both lectins. Optimum temperature for lectin 1 activity was 40-50 °C using blood types B and O, and 50 °C for lectin 2 using blood type O.

Using gel permeation chromatography, the molecular weights of lectins 1 and 2 were estimated to be 267 and 57 KD, respectively. From SDS-PAGE, lectin 1 was found to consist of six sub-units with apparent molecular weights of 32, 35.5, 39, 51.5, 55.5 and 59.5 KD. Lectin 2 was made up of sub-units with MW 15 and 17.5 KD, presumably of the $\alpha_2\beta_2$ type.

Lectins 1 and 2 were found to be glycoproteins containing 2.30% and 4.23% total sugars, respectively. The reducing sugar residues were identified to be glucose and galactose by high performance liquid chromatography (HPLC). Both lectins contained high amounts of aspartate and glutamate, a large proportion of which exist in the amide form. Lectin 1 was also rich in lysine and histidine.

INTRODUCTION

Lectins are originally known as phytohemagglutinins because in the past, they were mainly isolated from plants and were used to agglutinate red blood cells. In time, lectins have been found to be widely distributed in nature. Aside from plants, they appear to be present in microorganisms, invertebrates and even in some lower vertebrates. However, lectins are still predominantly found in legume seeds.

Because of the selective agglutination response of lectins and their ability to bind glycoconjugates, they are very useful in carbohydrate research where they are used as probes for identifying and mapping the sugars on the cell surfaces and as a specific reagent in affinity chromatography for the isolation of biomolecules containing sugars (Sharon, 1977; Sigma Chem. Co., 1993). They also find application in blood typing, in the elucidation of the structure of blood group substances; in the identification, diagnosis and typing of secretors; and even in the differential diagnosis of "polyagglutination" (Bird, 1959; Lis and Sharon, 1986).

Certain lectins are found to have promising uses in cancer and AIDS research (Inbar and Sachs, 1969; Favero et al., 1993). The ability of some lectins to react specifically with malignantly transformed cells is very useful in distinguishing these from normal cells as well as a means to understand the cell-surface changes that the cell undergoes during malignant transformation (Sharon, 1977). It has been reported that due to this very important property, some transformed cell-specific lectins are now being used as carriers of chemotherapeutic agents (Sharon and Lis, 1972).

Some lectins are found to be capable of mitogenesis *ie*, they have the ability to stimulate the transformation of lymphocytes (either thymus (T) or bone marrow (B) derived or both) and can be used as an aid in the diagnosis of the different types of leukemia (Lis and Sharon, 1973). Studies on mitogenic stimulation by lectins can also provide important information on the structure and organization of saccharides on the cell surface (Sharon, 1977).

In view of the numerous and promising applications of lectins in diverse fields of science, there is a need to evaluate new plant sources that display high lectin activities. This study was undertaken with the purpose of looking into the potential of the lablab bean as a lectin source.

The lablab bean, *Lablab purpureus* Linn cv. Highworth belongs to the *Leguminosae* family and is a wild relative of the native batao (*Dolichos lablab* Linn cv philippinensis). It is a fast-growing legume that is used as a forage. It can withstand severe grazing with 50% survival even after three close grazings. It grows well under humid and warm conditions but likewise shows good drought resistance due to deep root penetration

(Bogdan, 1977). Availability of the plant material is therefore not a problem because it is easy to grow.

A previous survey on lectin activity of seeds of eleven leguminous forage plant species have shown that the seeds of *Lablab purpureus* Linn cv. Highworth exhibited the highest lectin activity (Lacsamana, 1986). It is therefore noteworthy to further study the lectin found in the seeds of *L. purpureus* Linn cv. Highworth.

MATERIALS AND METHODS

Extraction of the Lectin

The seeds of *Lablab purpureus* Linn cv. Highworth were obtained from the International Rice Research Institute.

The dried seeds were dehulled manually, ground and defatted thrice in the cold using *n*-hexane at a ratio of 1:5 (w/v) for 10 minutes.

Phosphate buffered saline (PBS) at a concentration of 0.02 M and pH 7.2 was added to the defatted sample at a ratio of 5:1 (v/w) and stirred for 16 hours at 4°C, after which the mixture was filtered through a mira cloth. The crude PBS extract was clarified through centrifugation at 9,000 x *g* for 45 minutes at 4°C. The residue was discarded and the clarified crude PBS extract was stored at 0°C until use. The extract was subjected to hemagglutination assay and protein determination prior to purification.

Purification of the Lectin

The crude PBS extract was subjected to 90% ammonium sulfate saturation using Cooper's nomogram (1971). The precipitate was dissolved in a minimum amount of PBS and the ammonium sulfate was removed using a Sephadex G-25 desalting column. The resulting eluate was then subjected to ammonium sulfate fractionation.

Solid ammonium sulfate was added to the crude extract to give a 20% saturation. Precipitate formed was discarded and the corresponding centrifugate was treated with additional ammonium sulfate to give a 20 - 60% saturation. The resulting precipitate was collected by centrifugation at 9,000 x *g* for 45 minutes, followed by hemagglutination assay and protein determination Further purification was accomplished by passing the 20-60% ammonium sulfate fraction through Sephadex G-200 (superfine) column. Eluted fractions of 2.5 mL were monitored for absorbance at 280 nm and subjected to hemagglutination assay. All fractions that exhibited agglutination were pooled, concentrated using Polyethylene glycol (PEG) and then freeze-dried.

Hemagglutination Assay

Hemagglutination assays were done in plastic multi-well microtiter plates (Cooke's Engineering) using slightly outdated untrypsinized and trypsinized human blood (types A, B, O and AB) as well as goat and calf blood.

Inhibition of Agglutination

The effect of soluble sugar on the agglutination reaction was determined through the inclusion of the following standard sugars at 125, 250, 500 and 1000 mM concentration in the agglutination assay: D-glucose, D-fructose, D-mannose, D-galactose, L-fucose, D-arabinose, D-rhamnose, melibiose, inositol, galactosamine, lactose, sucrose, raffinose, mannosamine, glucuronic acid, maltose, xylose, cellobiose and methyl-α-D-mannopyranoside. Agglutination was inhibited when the erythrocyte cells formed a button at the bottom of the well.

Protein Determination

The protein content of the crude extract, the ammonium sulfate fractions and the concentrated Sephadex G-200 fractions was determined using the dye-binding method of Bradford with bovine serum albumin (BSA) as standard.

Characterization of Lectin

Effect of Temperature on Lectin Activity

Samples were incubated for 30 minutes in a water bath at different temperatures (20-70 °C) prior to hemagglutination assay using blood types B and O. For temperatures <20°C, the hemagglutination assay was done inside the cold room.

Effect of pH on Lectin Activity

The lectins were incubated with buffers of different pH for one hour prior to serial dilution and treatment with types B and O erythrocytes. The following buffers were used (Bates, 1964): KCl-HCl, pH 2; KHP-HCl, pH 3-4; KHP-NaOH, pH 5; KH_2PO_4-NaOH, pH 6-8; Tris-HCl, pH 9; $NaHCO_3$-NaOH, pH 10-11 and KCl-NaOH, pH 12-13.

Polyacrylamide Gel Electrophoresis (PAGE) in Non-denaturing Conditions

A. pH 8.8

Electrophoresis in a discontinuous polyacrylamide slab gel system was done on the lyophilized lectin 1 sample following the method of Laemmli (1970).

B. pH 4.3

The method of Reisfeld and his co-workers (1962) was adopted for the electrophoresis in non-denaturing conditions of lectin 2. The slab gel system used consisted of 4% polyacrylamide stacking gel (pH 6.8) and 12% polyacrylamide separating gel (pH 4.3).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The method of Laemmli (1970) for electrophoresis on polyacrylamide slab gel was followed. The following protein standards were used to determine the approximate molecular weight of the lectin sub-units: Set 1: [SDS-6H (Sigma)]: myosin (rabbit skeletal muscle) (200.0 KD); ß-galactosidase (*E. coli*) (116.25 KD); phophorylase B (rabbit muscle) (97.4 KD); bovine serum albumin (66.2 KD); ovalbumin (45.0 KD); carbonic anhydrase (29.0 KD). Set 2: [Dalton Mark VI (Sigma)]: bovine serum albumin (66.2 KD); ovalbumin (45.0 KD); pepsin (34.7 KD); trypsinogen (24.0 KD); b-lactoglobulin (18.4 KD); lysozyme (14.3 KD).

Molecular Weight Determination

The molecular weights of lectins 1 and 2 were estimated by gel permeation chromatography using superfine Sephadex G-200. The following standard proteins were used: b-amylase (200.0 KD); alcohol dehydrogenase (150.0 KD); bovine serum albumin (66.2 KD); carbonic anhydrase (29.0 KD); cytochrome C (12.4 KD).

Amino Acid Analysis

The determination of the amino acid composition of the purified lectin was conducted at the Bureau of Foods and Drugs, Alabang, Muntinlupa City.

Carbohydrate Analysis

High performance liquid chromatography was employed for the identification of sugars present in the lectin using the following standard sugars: D-xylose, D- galactose, D- glucose, D-mannose, D-fructose, sucrose, D-arabinose, maltose, lactose, melibiose, L-fucose, D-rhamnose, raffinose and stachyose.

Determination of Mitogenic Activity

Human peripheral lymphocytes were cultured according to the method of Toyoshima et al. (1970). A morphological assay was done (Soliven, personal communication) using Giemsa-stained preparations and viewed through HPO of a research microscope.

RESULTS AND DISCUSSION

Isolation and Purification of Lectins

The proteins were precipitated by saturating the crude extract with 90% ammonium sulfate. This was done to prevent possible protein denaturation in the crude extract and to keep the proteins in a more stable form. The resulting cream-gray precipitate was desalted using a Sephadex G-25 column before passing into the Sephadex G-200 column. The chromatogram that resulted showed two partially resolved but prominent peaks (Figure 1) having strong agglutinating activity. These peaks were resolved by subjecting the 20-60% ammonium sulfate fraction to gel permeation chromatography (Figure 2).

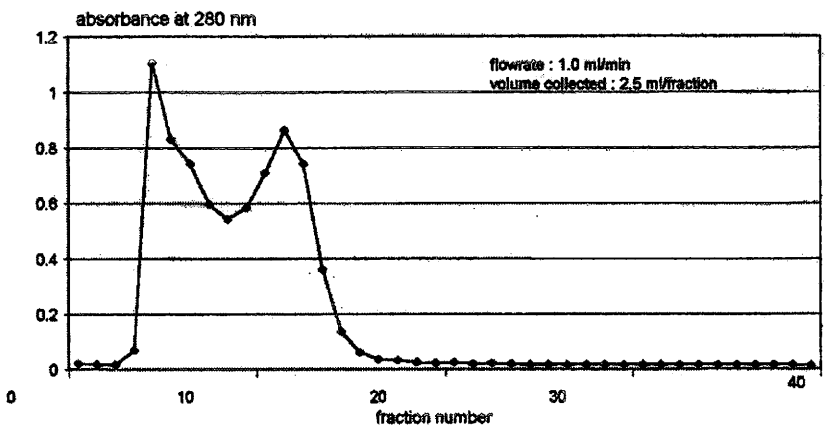

Figure 1. Gel permeation chromatography of 0-90% precipitate using Sephadex G-200.

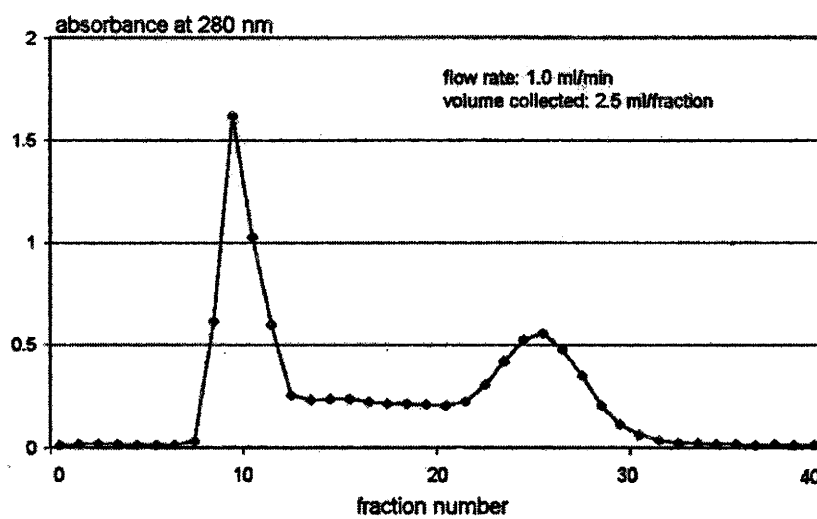

Figure 2. Gel permeation chromatography of the *L. purpureus* Linn cv Highworth lectins on superfine Sephadex G-200.

Purity of the lectins isolated from gel permeation chromatography was evaluated by native PAGE (10% gel) at pH 8.8 . The first and third peaks were designated as lectins 1 and 2, respectively. Lectin 1 showed a single band near the top of the separating gel, implying its high molecular weight. Lectin 2, did not show any band, presumably because of its very low protein content (Table 1) such that it may have fallen below the minimum concentration of protein capable of being detected by the staining reagent. The lectin 2 fractions were concentrated using polyethylene glycol (PEG), electrophoresed and a diffused band was seen just below the top of the separating gel. Similar observations were reported by Hapner and Robbins (1979) on sainfoin lectin and on jackfruit lectin by Ahmed and Chatterjee (1985) . These lectins reportedly behave differently from ordinary lectins because of their acidic character. It may very well be possible that lectin 2 is also an acidic protein and that the diffused band is the result of its precipitation at the alkaline pH of the polyacrylamide gel.

Table 1. Agglutinating activity and agglutination titer of lectins at varying degrees of purification.

Stage	Blood Type	Titer Value	Protein Content (mg/mL)	Agglutinating Activity[1]	Agglutinating Titer[2]
Crude Extract	A	256	2.66	96.24	10.39
	B	128	2.66	48.12	20.78
	O	256	2.66	96.24	10.39
	AB	256	2.66	96.24	10.39
0-90% $(NH_4)_2SO_4$ppt	A	256	2.62	97.71	10.23
	B	128	2.62	48.86	20.47
	O	256	2.62	97.71	10.23
	AB	256	2.62	97.71	10.23
20-60% $(NH_4)_2SO_4$ppt	A	256	2.56	100.0	10.00
	B	256	2.56	100.0	10.00
	O	512	2.56	200.0	5.00
	AB	256	2.56	100.0	10.00
Gel Chrom Lectin 1	A	32	0.884	36.20	27.62
	B	32	0.884	36.20	27.62
	O	32	0.884	36.20	27.62
	AB	32	0.884	36.20	27.62
Gel Chrom Lectin 2	A	16	0.0568	281.7	3.550
	B	16	0.0568	281.7	3.550
	O	32	0.0568	563.4	1.775
	AB	32	0.0568	563.4	1.775

[1]titer value/protein content in mg/mL [2]protein content in µg/mL/titer value

Most of the proteins present in the crude extract were recovered in the 20-60% ammonium sulfate fraction. Agglutinating activity increased as purification was achieved but the titer values decreased (Table 1). The protein content of the precipitates/isolates decreased at each step indicating that non-lectin proteins were being removed as purification was achieved while the specific activity of the lectin increased. Protein recovery after gel permeation chromatography was very low which suggests that the lectins comprised a very small fraction of the protein portion of the Highworth lablab bean seeds (Table 2).

A marked increase in the specific activity was noted at the last step of purification which indicates increased purity of the lectins isolated. This is supported by the high purification fold achieved especially by lectin 2.

Characterization of the Purified Lectins

Hemagglutination Assay

The purified lectins were found to be nonblood type specific because they agglutinated all types of human erythrocytes (A, B. O and AB). However, they are blood group specific since they did not agglutinate animal blood such as those of calf and goat . They can also be classified as complete lectins because they agglutinated untreated erythrocytes suspended in physiological saline solution.

The non-blood type specificity of the Highworth lablab bean lectins may be due to the possible presence of multiple binding sites in the lectins wherein N-acetyl-D-galactosamine, D-galactose and L-fucose may fit. These sugars are the determinants of types A, B and O, respectively.

Calf and goat erythrocytes were not agglutinated by both lectins because the lectin-specific receptor sites are in a cryptic form (Gordon et al., 1972). It is posiible that the animal red blood cells cannot interact strongly with the lectin due to lesser number of receptor sites (De Los Reyes, 1994) or the receptor sites may not be compatible with the lectin binding sites. Trypsin treatment had no effect on the goat and calf erythrocytes although this treatment renders the erythrocytes more susceptible to agglutination (Nicolson, 1971).

Inhibition of Agglutination

Hemagglutination by lectins could be inhibited or prevented by the addition of certain simple sugars. The inhibitory effect of sugars is attributed to their ability to compete for the binding sites on the lectin molecule thereby interfering with the attachment of the lectin to sugar units on the surface of the red blood cells.

Table 2. Purification of two lectins from *L. purpureus* Linn cv. Highworth seeds.

Stage	Blood Type	Titer Value	Total Vol. (mL)	Protein Content (mg/mL)	Total Protein (mg)	Agglutinating Activity	Total Agglutinating Activity	Specific Activity	Purification Fold	% Recovery
Crude Extract	A	256	100	2.66	266.0	96.24	9624	36.18	10.0	100
	B	128	100	2.66	266.0	48.12	4812	18.09	10.0	100
	O	256	100	2.66	266.0	96.24	9624	36.18	10.0	100
	AB	256	100	2.66	266.0	96.24	9624	36.18	10.0	100
0-90% $(NH_4)_2SO_4$ppt	A	256	95	2.62	248.9	97.71	9282	37.29	1.03	96.4
	B	128	95	2.62	248.9	48.86	4642	18.65	1.03	96.5
	O	256	95	2.62	248.9	97.71	9282	37.29	1.03	96.4
	AB	256	95	2.62	248.9	97.71	9282	37.29	1.03	96.4
20-60% $(NH_4)_2SO_4$ppt	A	256	28.2	2.56	72.19	100.0	2820	39.06	1.08	29.3
	B	256	28.2	2.56	72.19	100.0	2820	39.06	2.16	58.6
	O	512	28.2	2.56	72.19	200.0	5640	78.13	2.16	58.6
	AB	256	28.2	2.56	72.19	100.0	2820	39.06	1.08	29.3
Gel Chrom Lectin 1	A	32	1.0	0.884	0.884	36.20	36.20	40.95	1.13	0.376
	B	32	1.0	0.884	0.884	36.20	36.20	40.95	2.26	0.752
	O	32	1.0	0.884	0.884	36.20	36.20	40.95	1.13	0.376
	AB	32	1.0	0.884	0.884	36.20	36.20	40.95	1.13	0.376
Gel Chrom Lectin 2	A	16	1.0	0.0568	0.0568	281.7	281.7	4690	137	2.93
	B	16	1.0	0.0568	0.0568	281.7	281.7	4690	274	5.85
	O	32	1.0	0.0568	0.0568	563.4	563.4	9919	274	5.85
	AB	32	1.0	0.0568	0.0568	563.4	563.4	9919	274	5.85

Inhibition of Agglutination

Hemagglutination by lectins could be inhibited or prevented by the addition of certain simple sugars. The inhibitory effect of sugars is attributed to their ability to compete for the binding sites on the lectin molecule thereby interfering with the attachment of the lectin to sugar units on the surface of the red blood cells.

Based on hapten inhibition studies (Tables 3 and 4), some sugars were able to inhibit the agglutination activity of lectins 1 and 2. Agglutination of blood types B and O by lectin 1 was inhibited whereas those of blood types A and AB were not affected. On the other hand, agglutination of all blood types by lectin 2 was inhibited by certain sugars.

Even at low concentration (125 mM), the agglutination of blood type O by lectin 1 was strongly inhibited by D-fructose, D-glucose, maltose and methyl-α -D-mannopyranoside. Inhibition of agglutination was also affected by sucrose at 250 mM concentration. Agglutination of blood type B, on the other hand, by lectin 1 was fairly inhibited by 500 mM D-mannose and D-glucose.

Hapten inhibition of hemagglutination by lectin 2 showed that the interaction was prevented by the addition of sucrose, D-fructose, D-mannose, D-glucose or methyl-α-D-mannopyranoside regardless of blood type. Among the haptenic sugars, the most inhibitory was methyl-α-D-mannopyranoside which elicited a strong inhibition even at 125 mM. This was followed by D-mannose (125 mM for blood types A, B and O, and 250 mM for blood type AB) and D-glucose (250 mM for all blood types). Sucrose and D-fructose fairly inhibited the hemagglutination at concentrations of 500-1000 mM.

These results suggest that lectins 1 and 2 were able to bind the above mentioned sugars at varying degrees with lectin-sugar interactions strongest when the sugar was methyl-α-D-mannopyranoside. This may be because it exhibits one of the structural features of a sugar residue that is needed for sugar inhibition - a nonreducing end containing an unsubstituted methoxy group (Osawa, 1966).

Agglutination of blood type A by lectin 1 was not inhibited by any of the sugars tested. This indicates that N-acetyl-D-galactosamine residues on the surface of the cell membranes fit better on the binding sites of lectin 1 such that haptenic sugars were not able to dislodge them. In the case of lectin 1-blood type O interaction, the binding site involved may have additional combining regions such that the sites can better accommodate oligosaccharides like sucrose and maltose.

Table 3. Hapten inhibition study on lectin 1 from *L. purpureus* Linn cv. Highworth seeds.

Code	Sugar	Inhibition of Hemagglutination															
		A				B				O				AB			
		1000	500	250	125	1000	500	250	125	1000	500	250	125	1000	500	250	125
		(mM)*				(mM)				(mM)				(mM)			
1	sucrose	–	–	–	–	–	–	–	–	+	+/–	+/–	–	–	–	–	–
2	melibiose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	L-rhamnose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4	galactosamine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	methyl–α–D–mannopyranoside	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
6	inositol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	D–xylose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	D–mannose	–	–	–	–	+	+/–	–	–	–	–	–	–	–	–	–	–
9	D–fructose	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
10	lactose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11	mannosamine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12	D–fucose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13	D–galactose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14	maltose	–	–	–	–	–	–	–	–	+	+	+/–	+/–	–	–	–	–
15	D–glucose	–	–	–	–	+/–	+/–	–	–	+	+	+	+	–	–	–	–
16	glucuronic acid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
17	L–arabinose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
18	cellobiose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

*sugar concentration (+) inhibition (+/–) slight inhibition (–) no inhibition

Table 4. Hapten inhibition study on lectin 2 from *L. purpureus* Linn cv. Highworth seeds.

Code	Sugar	Inhibition of Hemagglutination															
		A				B				O				AB			
		1000	500	250	125	1000	500	250	125	1000	500	250	125	1000	500	250	125
			(mM)*				(mM)				(mM)				(mM)		
1	sucrose	+	+/–	–	–	+	+/–	–	–	+	+/–	–	–	+/–	–	–	–
2	D–fructose	+/–	+/–	–	–	+/–	+/–	–	–	–	–	–	–	+/–	+/–	–	–
3	D–galactose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4	L–arabinose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	D–mannose	+	+	+	+/–	+	+	+	+/–	+	+	+	+/–	+	+	+	–
6	L–rhamnose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	D–xylose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	glucuronic acid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9	melibiose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	D–fucose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11	mannosamine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12	galactosamine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13	maltose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14	lactose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
15	raffinose	–	–	–	–	+/–	+/–	–	–	–	–	–	–	–	–	–	–
16	inositol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
17	methyl–α–D–mannopyranoside	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	D–glucose	+	+	+/–	–	+	+	+/–	–	+	+	+/–	–	+	+	+/–	–

*sugar concentration (+) inhibition (+/—) slight inhibition (—) no inhibition

The same sugars were able to inhibit lectin 2-human erythrocyte binding implying that a common binding site maybe involved and that this binding site on lectin 2 has numerous clefts or grooves which can accommodate sugar residues with (a) equatorial -OH at C_3 and C_4 and a $-CH_2OH$ at C_6 and/or (b) a nonreducing end containing $-OCH_3$. These combining regions must also link to a sugar residue with bulky groups (*e.g.* fructose) or to one containing a nonreducing sugar end residue linked by a ß-glycosidic linkage (Makela, 1956).

Results of this study may explain the large difference in the agglutinating activities of lectins 1 and 2. Lectin 2 exhibits a very strong activity compared to lectin 1 maybe because its binding site can accommodate a wide variety of sugar residues and the resulting interaction may be very strong. Lectin 1, on the other hand, may have binding sites that are more or less specific for certain sugar configurations.

Effect of Temperature on Lectin Activity

Extreme temperatures (Table 5) completely destroyed the activity of both lectins. Lectin 1 activity reached its optimum at 40-50 °C when assayed using blood types B and O, with the former giving a higher activity. The highest activity of lectin 2 was noted at 50 °C using blood type O. Its activity rapidly declined at 60 °C and was totally lost at 70 °C .

Table 5. Effect of temperature on the titer values of two lectins from *L. purpureus* Linn cv. Highworth seeds.

Temperature (°C)	Lectin 1 Titer Value		Lectin 2 Titer Value	
	B	O	B	O
5	0	0	0	0
20	8	4	8	16
30	8	4	8	16
40	16	8	16	32
50	16	8	16	64
60	4	4	8	16
70	0	0	0	0

Refrigeration as well as subzero temperatures presumably inactivate the lectins. However, this phenomenon maybe reversible since increasing the temperature to some extent arrests this inactivation. Greater lectin activity at higher temperatures may be the result of increased mobility of receptor sites. Loss of lectin activity, however, may be attributed to the denaturation of lectins at much higher temperatures. Possible effects of temperature on the association-dissociation of sub-units in the lectin molecule may also be considered (Lis and Sharon, 1986).

Effect of pH on Lectin Activity

The influence of extreme pH values on the activity of the two lectins was noted using agglutination assays (Table 6). The activity of lectin 1 remained constant between pH 2 and 12 when assayed using blood type B but decreased twofold between pH 10 and 12 when blood type O was used. Lectin 2 activity was not affected at the range of pH 2-5 but decreased twofold from pH 6-12 using blood type O. When assayed using

Table 6. Effect of pH on the titer values of two lectins from *L. purpureus* Linn cv. Highworth seeds.

	Lectin 1		Lectin 2	
	Titer Value			
pH	B	O	B	O
2	8	8	8	16
3	8	8	8	16
4	8	8	8	16
5	8	8	8	16
6	8	8	8	8
7	8	8	8	8
8	8	8	8	8
9	8	8	8	8
10	8	4	4	8
11	8	4	4	8
12	8	4	4	8
13	0	0	0	0

blood type B, the effect of pH changes on the activity of lectin 2 followed the same pattern given by lectin 1 using blood type O. At pH 13, the activity of both lectins was destroyed .

Precipitation of lectins 1 and 2 was observed at pH 4-5 and 8-9, respectively indicating that the isoelectric point of the two lectins lies in these pH ranges. This also indicates that lectin 1 is a basic protein while lectin 2 is acidic in nature. It somehow supports the unusual behavior of lectin 2 on native PAGE.

Determination of Molecular Weight and Number of Sub-units

The molecular weights of the two lectins were estimated using gel permeation chromatography giving molecular weights of 267 KD and 57 KD for lectin 1 and 2, respectively.

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) showed a total of six bands for lectin 1 (Figure 3) and two bands for lectin 2 (Figure 4), none of which were identical. Lectin 1 was found to consist of three high and three low molecular weight sub-units. The approximate molecular weights of the six sub-units were calculated to be 32, 35.5, 39, 51.5, 55.5 and 59.5 KD for lectin 1 and 15 and 17.5 KD for lectin 2 .

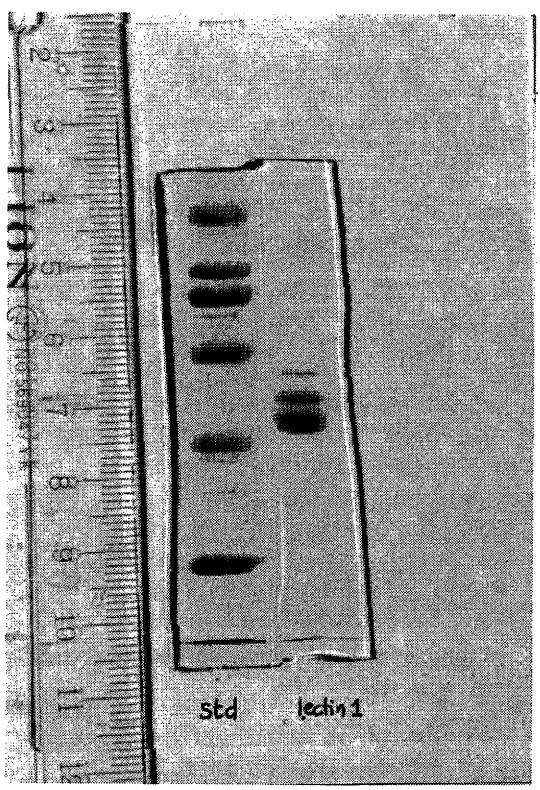

Figure 3. Sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) of lectin 1 isolated from the seeds of *L. purpureus* Linn cv Highworth.

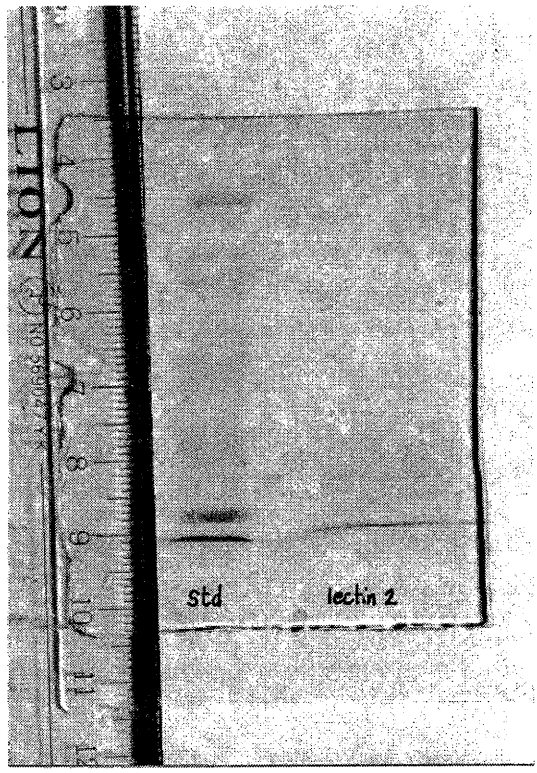

Figure 4. Sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) of lectin 2 isolated from the seeds of *L. purpureus* Linn cv Highworth.

which are identical. In the case of lectin 2, the sum of the molecular weights of the two sub-units (32.5 KD) is only half of the estimated molecular weight (57 KD) signifying that the actual number of sub-units is twice than what was observed. This is probably because each of the two bands seen in SDS-PAGE represents two sub-units having similar molecular weights. Lectin 2 can therefore be classified under the $\alpha_2\beta_2$ type.

Amino Acid Analysis

The amino acid composition of lectins 1 and 2 is given in table 7. Both lectins were found to be rich in aspartate and glutamate while lectin 1 also contained high amounts of lysine and histidine. Lectin 2 contained negligible amount of valine and phenylalanine. Acid hydrolysis of both lectins prior to amino acid analysis resulted in the release of 151 and 682 μg NH_3/mL from lectins 1 and 2, respectively. This indicates that a large proportion of the aspartic and glutamic acid residues in the lectin molecules are present in the amide form, *i.e.* as asparagine and glutamine, respectively.

Table 7. Amino acid composition of two lectins from *L. purpureus* Linn cv. Highworth seeds.

Amino Acid	Lectin 1 Percentage	Lectin 2 Percentage
Aspartate	10.17	15.46
Threonine	2.85	4.21
Serine	5.27	6.51
Glutamate	10.93	19.60
Glycine	2.79	5.64
Alanine	2.82	5.79
Cysteine	3.50	3.84
Valine	1.61	0.33
Methionine	1.44	2.69
Isoleucine	3.22	7.31
Tyrosine	12.35	8.47
Phenylalanine	2.34	0.93
Histidine	11.23	5.08
Lysine	26.33	8.65
Arginine	2.15	5.46
NH_3	151 µg/mL	682 µg/mL

Carbohydrate Analysis

High performance liquid chromatography analysis of the sugar hydrolysates from both lectins gave a broad band each. Comparison of the retention times of the unknown peaks with those of the standard sugars together with spiking of the unknown peaks with standard sugars (Figure 5 and 6) revealed the reducing sugar components to be glucose and galactose.

Quantitative analysis of the sugar components revealed that lectin 1 contained 1.53% glucose, 0.74% galactose and 0.04% non-reducing sugars while lectin 2 contained 0.39% glucose, 3.77% galactose and 0.09% non-reducing sugars.

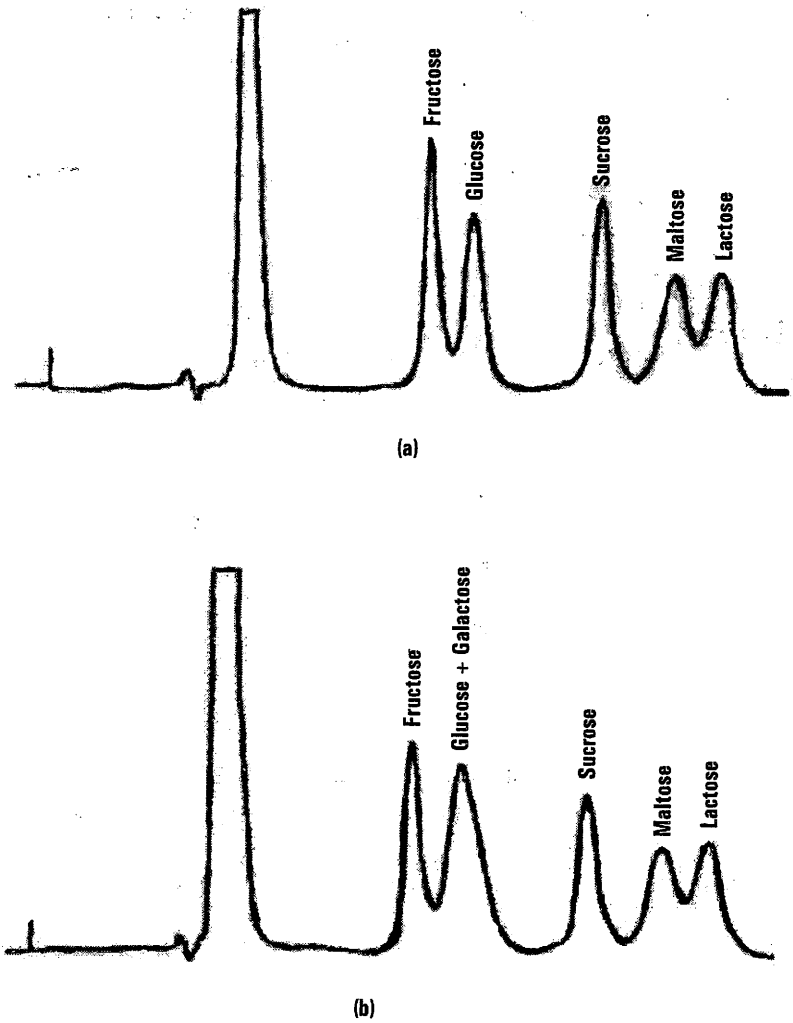

Figure 5. HPL Chromatograms of mixtures of standard sugars (a) without and (b) with galactose.

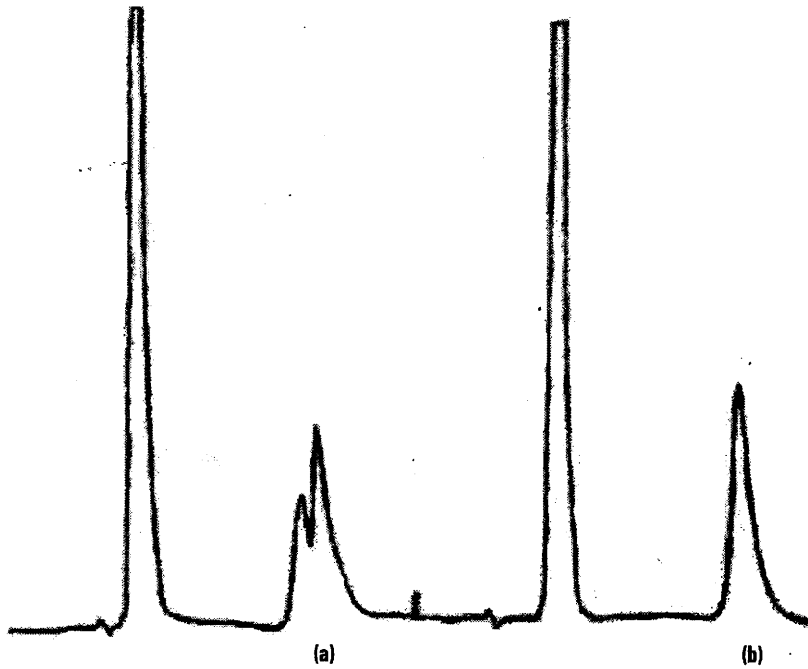

Figure 6. HPL Chromatograms of lectin 2 spiked with 10 μl (a) glucose and (b) galactose (0.5 cm/min chart speed 32x attenuation, 0.5 mL/min flow rate; $CH_3CN:H_2O$ (75:25 v/v)).

Determination of Mitogenic Activity

Both lectins from the Highworth lablab bean have mitogenic activity (Figure 7). High percentages of lymphoblasts were observed after 36 hours of incubation. Further incubation resulted in the reversion of postmitotic cells to small lymphocytes as shown by the rapid decline in the number of lymphoblasts noted after 72 hours.

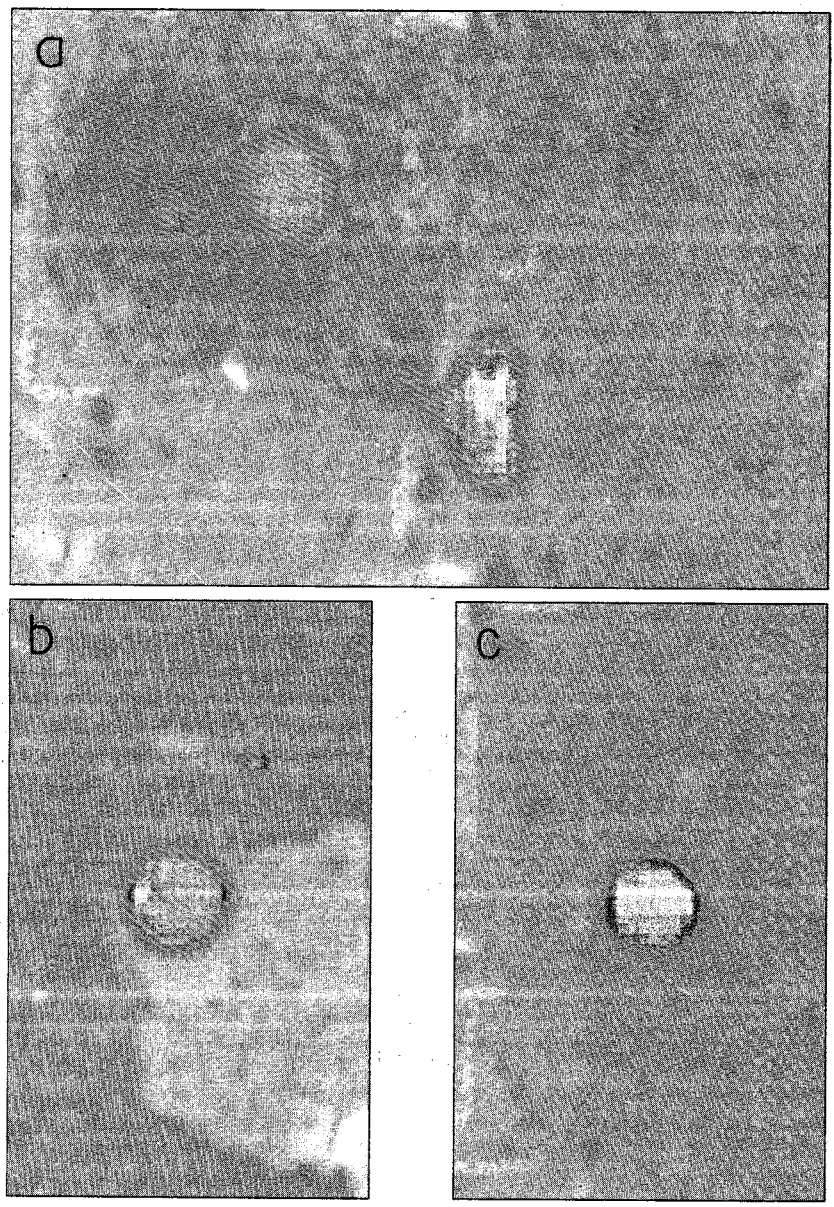

Figure 7. Photomicrographs of lymphocytes stimulated by a) *Phaseolus vulgaris* lectin (PHA) b) lectin 1 c) lectin 2. A lymphocytes in the process of cell division in lower a) plate (40x).

SUMMARY AND CONCLUSION

Two lectins were isolated and purified from the seeds of *Lablab purpureus* Linn cv Highworth through protein extraction using PBS pH 7.2, ammonium sulfate fractionation and gel permeation chromatography on superfine Sephadex G-200. The homogeneity of the lectins was determined by native polyacrylamide gel electrophoresis at pH 8.8 (lectin 1) and pH 4.3 (lectin 2).

Both lectins were found to be non-blood type specific because they agglutinated human blood types A, B, O and AB but were classified as blood group specific because they did not agglutinate normal and trypsin-treated calf and goat erythrocytes. Because they were able to agglutinate untreated erythrocytes, they fall into the category of complete lectins.

Hapten inhibition study on both lectins revealed that lectin 1 activity was inhibited by D-fructose, D-glucose, maltose, sucrose and methyl-α-D-mannopyranoside using blood type O; and by D-glucose and D-mannose using blood type B. On the other hand, lectin 2 activity was inhibited by the addition of D-fructose, D-mannose, D-glucose and methyl-α-D-mannopyranoside regardless of blood type. Among the haptenic sugars, methyl-α-D-mannopyranoside elicited the strongest inhibition. Agglutination of blood types A and AB by lectin 1 was not inhibited by any of the sugars used.

Extreme temperatures completely destroyed the activity of both lectins. Optimum temperature for lectin 1 activity using blood types B and O was found to be between 40-50 °C, while highest activity for lectin 2 was noted at 50 °C using blood type O.

Effects of pH on the activity of lectins 1 and 2 were investigated through hemagglutination assay using blood types B and O. The activity of lectin 1 remained constant from pH 2-12 using blood type B although there was a twofold decrease in its activity at pH 10-12 using blood type O. Lectin 2 activity was unaffected from pH 2-5 but decreased twofold from pH 6-12 using blood type O. At pH 13, the activity of both lectins was destroyed.

Precipitation of lectins 1 and 2 were noted at pH 4-5 and 8-9, respectively, indicating that their isoelectric point lie in these ranges. It also indicates that lectin 1 is basic while lectin 2 is acidic.

Using gel permeation chromatography, the molecular weights of lectins 1 and 2 were estimated to be 267 and 57 KD, respectively. From SDS-PAGE, lectin 1 was found to consist of six sub-units of apparent molecular weights of 32, 35.5, 39, 51.5, 55.5 and 59.5 KD. Lectin 2 on the other hand, was made up of sub-units weighing 15 and 17.5 KD, presumably of the $\alpha_2\beta_2$ type.

Lectin 2 contained relatively high amounts of aspartate and glutamate while lectin 1 is rich in lysine and histidine. A large proportion of the acidic amino acids reported may actually be in the amide form as adjudged by the high concentration of ammonia liberated during acid hydrolysis of the lectins.

Carbohydrate analyses of both lectins showed that both are glycoproteins and that glucose and galactose are their major carbohydrate components. Lectin 1 contained 1.53% glucose, 0.74% galactose and 0.04% non-reducing sugars whereas lectin 2 contained 0.39% glucose, 3.77% galactose and 0.09% non-reducing sugars.

Resting human peripheral blood lymphocytes were stimulated by lectins 1 and 2 to enlarge into blast cells after 24 hours. Highest percentages of transformed cells were noted after 36 hours of incubation, after which a rapid decline was observed signifying the reversion of lymphoblasts into small lymphocytes after mitosis.

RECOMMENDATIONS

Due to the numerous haptenic sugars noted, it is possible that lectins 1 and 2 exist as isolectins. However, the methods employed in this research work were not able to establish their presence. It is therefore recommended that further research be done in this area. Because of the results of the hapten inhibition study, several types of affinity carriers may be examined for the easy isolation and purification of these lectins.

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Antimicrobial Resistance Patterns of *Acinetobacter antitratus* from Cases of the Philippine General Hospital and the Conjugative Transferability of the Resistance

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ABSTRACT

Acinetobacter anitratus isolates from patients of the Philippine General Hospital were studied for their antimicrobial or drug resistance patterns and the transferability of the resistance to Escherichia coli SF-800 by conjugation. Twenty four of the 25 isolates were found to be resistant to at least one of the following test drugs, namely: ampicillin with sulbactam, amikacin, gentamicin, tobramycin, trimethoprim-sulfamethoxazole and nalidixic acid. Eighteen of the 25 isolates showed multiple resistance, some to as many as five drugs. Results of the conjugation studies showed that all isolates tested were able to transfer their resistance to E. coli SF-800. Three isolates transferred all of the resistance tested while the other ten isolates transferred only some of their resistance.

INTRODUCTION

Nosocomial or hospital-acquired infections are transmitted to patients either by hospital personnel or by other patients. They can be acquired through surgical procedures, bladder catheters, endotracheal tubes, intravenous fluids and other equipment (Joklik et al., 1984). Infection and colonization with *Acinetobacter sp.* occur when the organism is introduced into traumatic wounds particularly if there has been soil or water contamination. *Acinetobacter sp.* is also found in the normal flora of the skin and mucous membranes of humans. It has also been isolated from hospital walls and fixtures (Zembrzuska-Sadkowska, 1995). Although it possesses very little invasive activity, it had been found to be an etiologic agent of pneumonia and urinary tract infections (Finegold and Baron, 1986). Likewise, it has recently emerged as an important nosocomial pathogen because of its resistance to the majority of commonly-used antimicrobial agents (Triantafilo et al., 1997; Sing and Yeo, 1996; Roussel-Delvallez et al., 1996).

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One of the serious problems faced today in the treatment of diseases is the development of bacterial resistance to drugs used. Drug resistance can be inherent in the bacteria or caused by chromosomal mutation to resistance. More importantly, it can also be caused by extrachromosomal genetic elements called R plasmids or resistance plasmids. R plasmids usually code for resistance to multiple drugs. Some plasmids are transferable to other bacteria belonging to the same or to different genera and species through conjugation. This results in the conversion of the drug sensitive recipients to resistant strains, usually with multiple resistance.

This study aimed to determine the response of nosocomial *A. anitratus* to commonly-used antimicrobial agents and to determine the conjugative transferability of the resistance characteristics in these isolates to *E. coli.*

MATERIALS AND METHODS

A. anitratus isolated from patients of the Philippine General Hospital from September to November, 1996 were studied. The isolation and identification were done by the staff of the Bacteriology Section of the Department of Laboratories of the hospital. Twenty five isolates were tested for their susceptibility to the following drugs, namely: ampicillin with sulbactam [10 micrograms (µgs)], amikacin (30 µgs), gentamicin (10 µgs), tobramycin (10 µgs), trimethoprim-sulfamethoxazole (1.25 µgs, 23.75 µgs) and nalidixic acid (30 µgs). *E. coli* SF800 which is $Lac^{+}Res^{-}$ was used as the recipient for the conjugative transferability.

The drug susceptibility patterns of the isolates were determined using the disc-agar diffusion method modified from that of Bauer *et al.* (1966). The inocula used were four-hour cultures in tryptic soy broth (TSB) prepared from 18-hour TSB cultures. Turbidity of the isolates was adjusted to equal that of 0.5 MacFarland standard to approximate an inoculum concentration of 1.5×10^{8} cells ml^{-1}. The isolates were plated on Mueller-Hinton II agar. The diameters of the zones of inhibition were measured to the nearest millimeter and interpreted with the use of the zone diameter interpretative chart of the National Committee on Clinical Laboratory Standards (N.C.C.L.S.).

Conjugation was carried out using a modification of the method of Sinclair *et al.*,(1981). Of the 25 isolates, thirteen isolates which were multiply resistant to the test drugs were used as donors in the conjugation studies. Fifty microliters (uls) and 200 uls of donor and recipient cells, respectively, were inoculated in tryptic soy agar plates using the spread plate method. The plates were incubated at 37°C for four to six hours. Sterile saline solution with a volume of 1.5 ml was added to each plate after which the growth was harvested. One hundred uls of the growth mixture were inoculated into Mac Conkey agar plates containing each of the following: ampicillin with sulbactam (10 µgs/ml), amikacin (30 µgs/ml), gentamicin (10 µgs/ml), tobramycin (10 µgs/ml), and trimethoprim sulfamethoxazole (1.25 µgs, 23.75 µgs/ml). The resulting *E. coli* transconjugants were lactose fermenters showing resistance to the test drug(s). These were differentiated from the donor, *A. anitratus* which were non-lactose fermenters and from *E. coli* SF 800 which did not receive the resistance genes. The latter did not grow on the selective Mac Conkey agar plates with the drugs. Using the Modified Kirby-Bauer diffusion method, the responses of the transconjugant suspects to the test drugs were confirmed.

The 18-hour cultures of *A. anitratus* donors, and *E. coli* SF 800 were also inoculated in selective plates with the individual antibiotics to serve as controls. This was done to check for the emergence of spontaneous mutants to resistance and also to check the efficacy of the drugs in the plates used.

RESULTS AND DISCUSSION

The resistance phenotypes of the *A. anitratus* isolates are shown in Table 1. The results show that out of the 25 isolates, 24 (96%) were resistant to at least one drug, with 18 out of the 24 (75%) being multiply-resistant. Four (16 %) of these were resistant to two drugs and five (20 %) isolates were resistant to three drugs. Six (24 %) isolates were resistant to four drugs and three (12 %) were resistant to five drugs. Results correlate well with those of studies done in other countries which also show multiple drug resistance in most if not all of the isolates tested (Triantafilo et al., 1997; Shi-Zy et al., 1996; Roussel-Delvallez et al., 1996; Ling et al., 1996; Sing and Yeo, 1996; De and Deodhar, 1995). Literature search done by the authors did not yield published data on the drug resistance profiles of local *Acinetobacter* isolates.

Table 1 Resistance phenotypes of the *Acinetobacter anitratus* isolates from patients of the Philippine General Hospital.

Isolate Number	Resistance Phenotype
1	NA, GM, TS, TN, AN
2	SAM, NA, GM, TS
3	NA, GM, TS, TN
4	NA, GM, TS, TN, AN
5	SAM, NA, TS
6	NA, TS
7	NA, GM, TS
8	NA, GM, TS
9	NA
10	NA, GM, TS
11	NA, TS
12	NA
13	NA
14	NA, TS, TN
15	NA, GM, TS, TN
16	NA, GM, TS, TN
17	NA, GM, TS, TN, AN
18	NA
19	NA, TS
20	NA
21	SAM, NA, TS
22	NA
23	NA, GM, TS, TN
24	sensitive
25	NA, TS

SAM - ampicillin with sulbactam
AN - amikacin
GM - gentamicin
NA - nalidixic acid
TN - tobramycin
TS - trimethroprim-sulfamethoxazole

Table 2 shows that 24 (96%) of the 25 isolates tested were resistant to nalidixic acid. The study of Ferreira et al. (1984) shows that 48% of the *Acinetobacter* isolates tested were still sensitive to the drug in contrast to the results of the present study where only 4% of the isolates (one isolate) was sensitive to it. Amikacin was shown to be the antibiotic to which most isolates (84%) were sensitive. This was followed by sensitivity to ampicillin/sulbactam (80%).

Table 2: Response of the 25 isolates of *Acinetobacter anitratus* from patients of the Philippine General Hospital to the different test antibiotics.

Antibiotics	Susceptible Isolates		Resistance Isolates	
	Number	%	Number	%
SAM (10 ugs)	20	80	4	16
NA (30 ugs)	1	4	24	96
GM (10 ugs)	14	56	11	44
TS (25 ugs)	7	28	18	72
TN (10 ugs)	17	68	8	32
AN (30 ugs)	21	84	3	12

ugs - micrograms
SAM - ampicillin with sulbactam
AN - amikacin
GM - gentamicin
TN - tobramycin
TS - trimethroprim-sulfamethoxazole
NA - nalidixic acid

Ferreira et al. (1984) also showed that the *Acinetobacter* isolates tested were most sensitive to amikacin. Ling et al. (1996) found that amikacin was "reliably active" against the organism. In the study of Gales et al. (1996) however, only 16% of the isolates tested was sensitive to amikacin. Results of Urban et al. (1993) and Gales et al. (1996) among others, also show that *A. anitratus* was susceptible to ampicillin with sulbactam. Sulbactam, a beta-lactamase inhibitor is directed against plasmid-mediated enzymes and various extended-spectrum enzymes. Sulbactam is also active against some of the chromosomally-mediated enzymes and is active against *Acinetobacter* and *Bacteroides* (Williams, 1997). Resistance to trimethoprim-sulfamethoxazole, gentamicin and tobramycin were shown by 72%, 44% and 32% of the isolates, respectively.

Data on the transferability of the drug resistance to *E. coli* SF800 through conjugation show that all of the thirteen isolates transferred their resistance to the recipient (Table 3). Of these thirteen isolates, three effected complete transfer (i.e., all resistance tested were transferred). The other isolates transferred only some of their

Table 3 Resistance transferred and not transferred to *Escherichia coli* SF 800 from *Acinetobacter anitratus* isolates from patients of the Philippine General Hospital after conjugation.

Isolate Number	Resistance transferred	Resistance not transferred	Interpretation
SF-800 + isolate number 1	GM, TN, TS	SAM, AN	Partial transfer
SF-800 + isolate number 2	GM, TN, TS	SAM, AN	Partial transfer
SF-800 + isolate number 3	GM, TN	TS	Partial transfer
SF-800 + isolate number 4	AN, TN, TS	GM	Partial transfer
SF-800 + isolate number 5	TS	SAM, TN	Partial transfer
SF-800 + isolate number 7	GM, TS	none	Complete transfer
SF-800 + isolate number 8	GM, TS	none	Complete transfer
SF-800 + isolate number 14	TN	TS	Partial transfer
SF-800 + isolate number 15	TS	GM, TN	Partial transfer
SF-800 + isolate number 16	GM, TN, TS	none	Complete transfer
SF-800 + isolate number 17	GM	AN, TN, TS	Partial transfer
SF-800 + isolate number 21	TS	SAM	Partial transfer
SF-800 + isolate number 23	GM, TS	TN	Partial transfer

SAM - ampicillin with sulbactam
AN - amikacin
GM - gentamicin
TN - tobramycin
TS - trimethoprim-sulfamethoxazole

resistance. Resistance to nalidixic acid, on the other hand has been reported to be chromosomally mediated (Joklik et al., 1984), so its transfer was not expected. The same result was obtained in the study.

Local studies also show the conjugative transfer of drug resistance from nonfermentative, gram negative bacilli like *Pseudomonas aeruginosa* (Cabrera et al., 1997), and from fermentative, gram negative bacilli like *Vibrio cholera* (Cabrera, 1994), *Salmonella* spp. (Cabrera, 1987) and *E. coli* (Hernandez and Raymundo, 1989; Cabrera, 1988). The results are of significance to public health. When bacteria

become resistant to drugs by the acquisition of R-plasmids, they acquire a fast recovery rate, are selectively filtered out for survival and are disseminated in the environment, most especially in an environment with heavy usage of antibiotics such as in a hospital setting. Considering that R plasmids are also often transferable even to those belonging to a different family, such as between *Acinetobacter sp.* and *E. coli,* their presence in bacteria increases the chance of nosocomial infections caused by multiple drug resistant strains (Saunders, 1984). This definitely results in difficult therapeutic problems.

RECOMMENDATION

Considering the rapid emergence of *Acinetobacter* sp. as a nosocomial pathogen in other countries, many isolates of which are multiple drug resistant strains, and the finding that its resistance is transferable to other bacteria belonging to a different family, it is recommended that more intensive monitoring of cases and more extensive study of local *Acinetobacter* isolates, in particular, their drug resistance characteristics, be pursued.

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Leaf Morpho-Anatomy of Six Species of *Pinus* L. (Abietaceae)

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ABSTRACT

The leaf morphology of six species of Pinus were studied and compared. The leaf characteristics useful in distinguishing the species are shape in transection, stomatal frequency, stomatal index, length of epidermal cells, polar subsidiary cells, guard cells, nature of hypodermal cells, mesophyll cells, number and distribution of resin canals and vascular bundle. A key based on these morpho-anatomical features is presented.

INTRODUCTION

Investigations dealing with the morphology and anatomy of conifer leaves are of limited nature. Laubenfels (1953) described a wide range of morphological forms in the conifer leaves. Emphasizing the value of the leaf structure in the taxonomic studies, Carlquist (1961) points out that no generic monograph can be complete without the study of leaf anatomy. Amongst the conifers *Pinus* is one of the largest genus and according to figure given by Mirov (1967) 105 species are included in this genus. Investigations dealing with the anatomical structure in conifer leaves are of limited nature, Coulter & Rose (1886), Shaw (1914), Florin (1931), Konar (1960), Mirov (1967), Maheshwari & Konar (1971), Esau (1965), Carde (1978), Konar & Ramchandani (1958). The only detailed microscopical study of the needle leaves of *Pinus* has been made among others by Sutherland (1933), Huber (1947), Strasburger (1891), Doi & Morikawa (1929), Galo & Amoroso (1993) and Chaturvedi (1990, 1993a, 1993b, 1995).

The present paper provides a comparative accout of epidermal tissue and anatomical features in the needle leaves of six species of *Pinus* viz. *P. densiflora, P. echinata, P. griffithii, P. koriensis, P. sylvestris* and *P. tabulaeformis.* Since such type of comparative study has not yet been undertaken and scattered literature is present in the genus *Pinus* hence further extensive study on the morpho-anatomical features need to be conducted.

MATERIALS AND METHODS

The herbarium of *Pinus* shoots were obtained from Hamburg West Germany. Epidermal tissues were isolated by maceration in Schulze's fluid (Conc. HNO_3 +$KClO_3$) and were mounted in safranin glycerine jelly. Needle leaves were cut at 10-15 mm by microtome and free hand sections. Sections were stained using safranin and fast green combination and mounted in canada balsam. Photographs were taken in Leitz Biomed.

The ratio of epidermal cells and stomata in the same unit area is defined as stomatal index. This ratio is denoted by the following expressions

$$I = \frac{S}{E+S} \times 100$$

where, I = stomatal index
S = stomatal number in unit area
E = number of epidermal cells in the same unit area
The frequency of stomata per square millimeter was determined on the basis of an average of about twenty five readings in each case.

RESULTS AND DISCUSSION

Surface view of Epidermal Tissue (Pl. 1 and 2)

The needle leaves are bifacial in *P. densiflora, P. sylvestris,* while trifacial in *P. echinata, P. griffithii, P. koriensis* and *P. tabulaeformis.* The epidermal cells of six species are sinuous walled, longitudinally elongated, rectanguloid to polygonoid. Size of epidermal cells is 160-140 μm in length x 100-80 μm in width in *P. densiflora.* 240-200 μm in length x 70-60 μm in width in *P. griffithii,* 200-180 μm in length x 170-150 μm in width in *P. koriensis,* 100-80 μm in length x 60-40 μm in width in *P. sylvestris* and 140-120 μm in length and 80-40 μm in width in *P. tabulaeformis.*

Lateral walls straight end walls oblique or transverse, cuticle of all faces showing narrow stomatiferous bands alternating with 4 to 8 cells wide non-stomtiferous bands.

Stomata haplocheilic surrounded usually by 6-8 subsidiary cells but sometimes the number may vary from 4-6. Guard cells measure 30mm in length x 16 μm in width in *P. densiflora,* 35 μm in length x 20 μm in width in *P. echinata,* 40 μm in length x 16 μm in width in *P. griffithii* and *P. koriensis,* 32 μm in length x 23 μm in width in *P. sylvestris* and 35 μm in length x 26 μm in width in *P. tabulaeformis.*

The polar subsidiary cells measure 62 μm in length x 42 μm in width in *P. densiflora, P. sylvestris* and *P. tabulaeformis* 60 μm in length x 40 μm in width in *P. echinata, P. koriensis* and 66 μm in length x 44 μm in width in

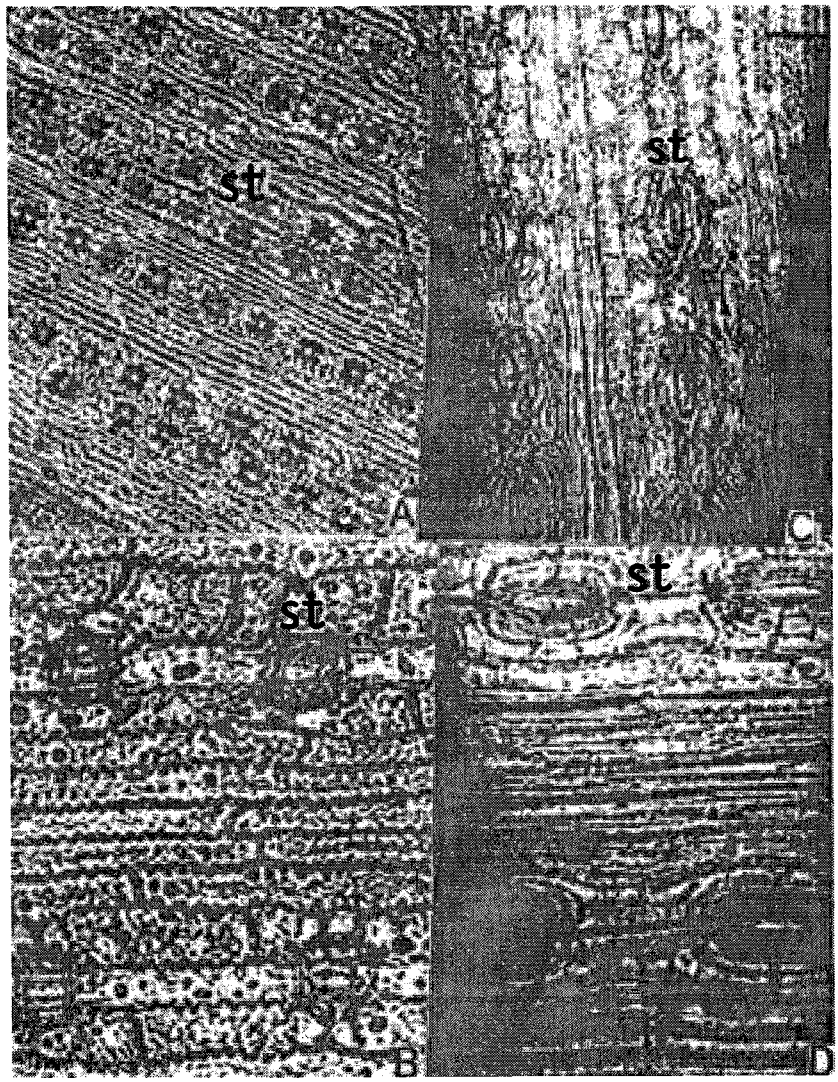


Plate 1

st - stomata

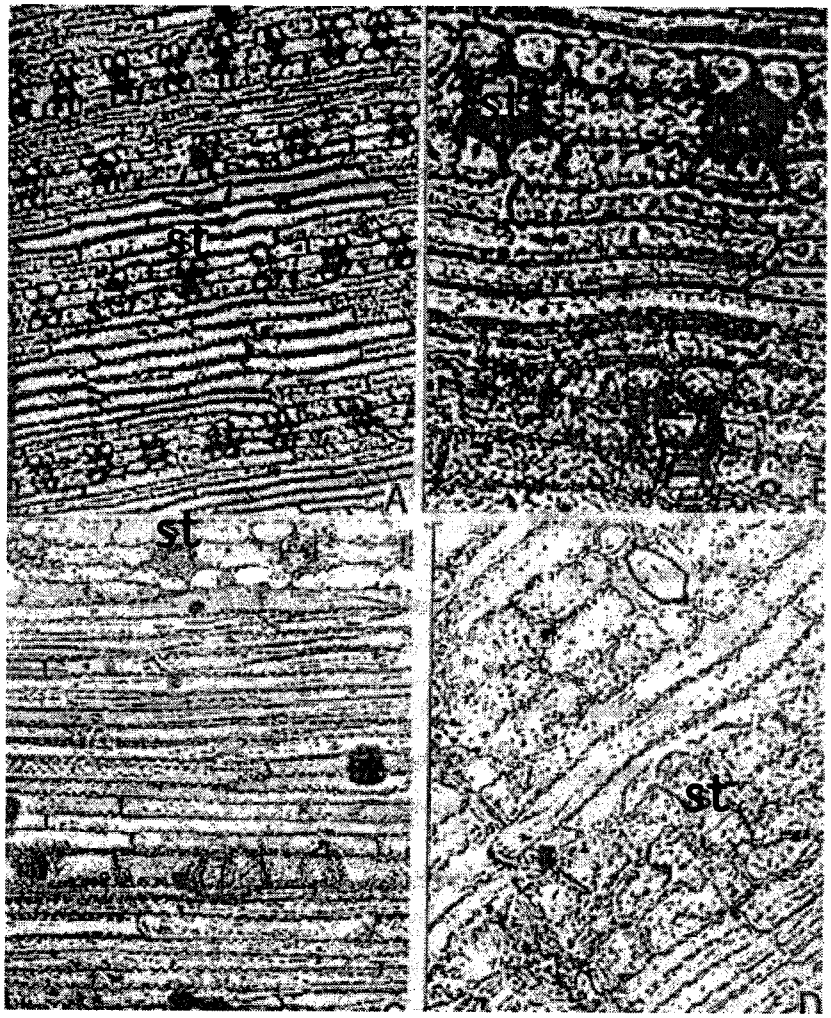
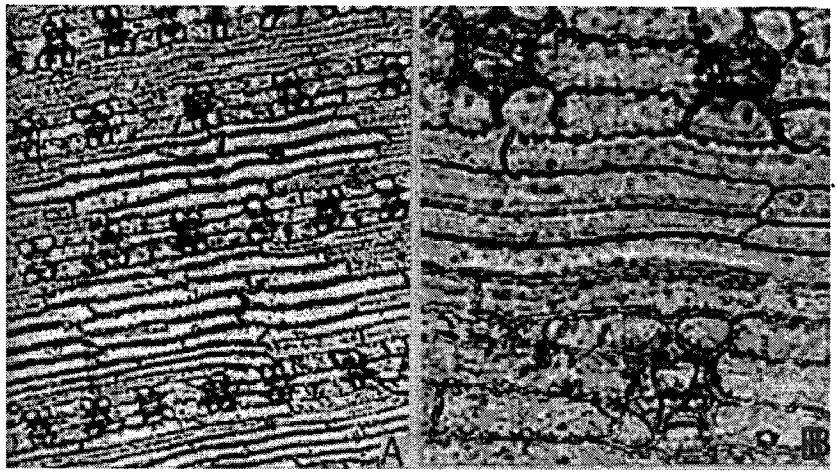


Plate 2

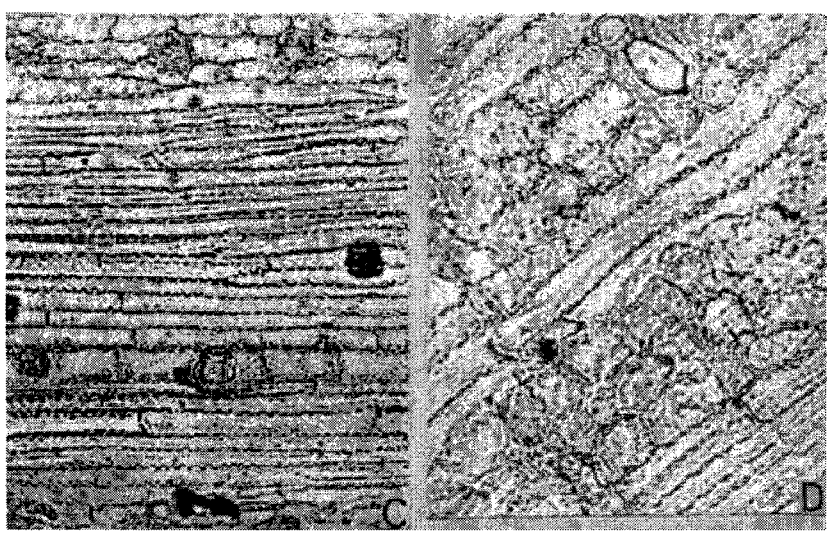
st - stomata



Pinus koriensis

Figure A. Epidermal cells sinuous walled. Cuticle showing stomatiferous and non stomatiferous bands. Stomata arranged in longitudinal rows x 200.

Figure B. Portion in Figure A magnified to show haplocheilic stomata, guard cells surrounded by 6 subsidiary cells x 320.



Pinus tabulaeformis

Figure C. Epidermal cells sinuous walled. Cuticle showing stomatiferous and non stomatiferous bands. Stomata arranged in longitudinal rows x 160.

Figure D. Portion in Figure A magnified to show haplocheilic stomata, guard cells surrounded by 6 subsidiary cells x 320.

P. griffithii.

The stomatal frequency is 86 st/mm^2 in *P. densiflora,* 88 st/mm^2 in *P. echinata,* 79 st/mm^2 in *P. griffithii,* 86 st/mm^2 in *P. koriensis* 94.2 st/mm^2 in *P. sylvestris* and 92.5 st/mm^2 in *P. tabulaeformis.* The stomatal index is 89.5 in *P. densiflora,* 90.8 in *P. echinata* and *P. griffithii,* 92 in *P. koriensis,* 96 in *P. sylvestris* and 94 in *P. tabulaeformis.*

Leaf Anatomy (Pls 3, 4, 5, 6, 7 & 8)

A. Shape of needle in Transection

Transection of the needle leaves show crescent shape outline in *P. densiflora,* 'T' shape in *P. echinata,* 'T' or wedge shaped in *P. griffithii,* traingular in *P. koriensis* 'D' shape in *P. sylvestris* and bowl shaped in *P. tabulaeformis.*

B. Epidermal Tissue

Epidermal cells are isodiametric, 22µm in length x 18µm in width in *P. densiflora,* tangentially elongated 22µm in length x 17.5µm in width in *P. echinata,* tangentially elongated 16µm in length x 14µm in width in *P. griffithii,* nearly isodiametric 22.5µm in length x 18µm in width in *P. koriensis,* radially elongated 23.2µm in length x 11.6µm in *P. sylvestris* and tangentially elongated, 22µm in length x 19µm in width in *P. tabulaeformis.*

C. Hypodermis

Hypodermis usually single layered, cells tangentially elongated in *P. densiflora, P. echinata, P. griffithii, P. koriensis, P. tabulaeformis* and 2-3 layered polygonal cells in *P. sylvestris.*

D. Mesophyll

Mesophyll composed of distinctly stellate cells showing prominent peg like ingrowths and irregular outline in all the species. The number of layers vary. It is 1-2 layered in *P. echinata, P. griffithii* and *P. koriensis,* 2-3 layered in *P. tabulaeformis* and 3-5 layered in *P. densiflora* and *P. sylvestris.*

E. Number and Distribution of Resin Canals

The number of resin canals differ. It is 3 in *P. echinata, P. griffithii* and *P. koriensis,* 4 in *P. densiflora,* 4-6 in *P. sylvestris* and *P. tabulaeformis.*

F. Vasular bundle

Vascular bundle centrally located which shows formation of secondary xylem and phloem. Its nature varies being one in *P. echinata, P. griffithii* and *P. koriensis,* two in *P. densiflora, P. sylvestris* and *P. tabulaeformis.*

G. Transfusion Tissue

Transfusion tissue is well developed consisting of relatively large cells around the phloem, thick or thin walled cells opposite the xylem in all the species except in *P. densiflora.*

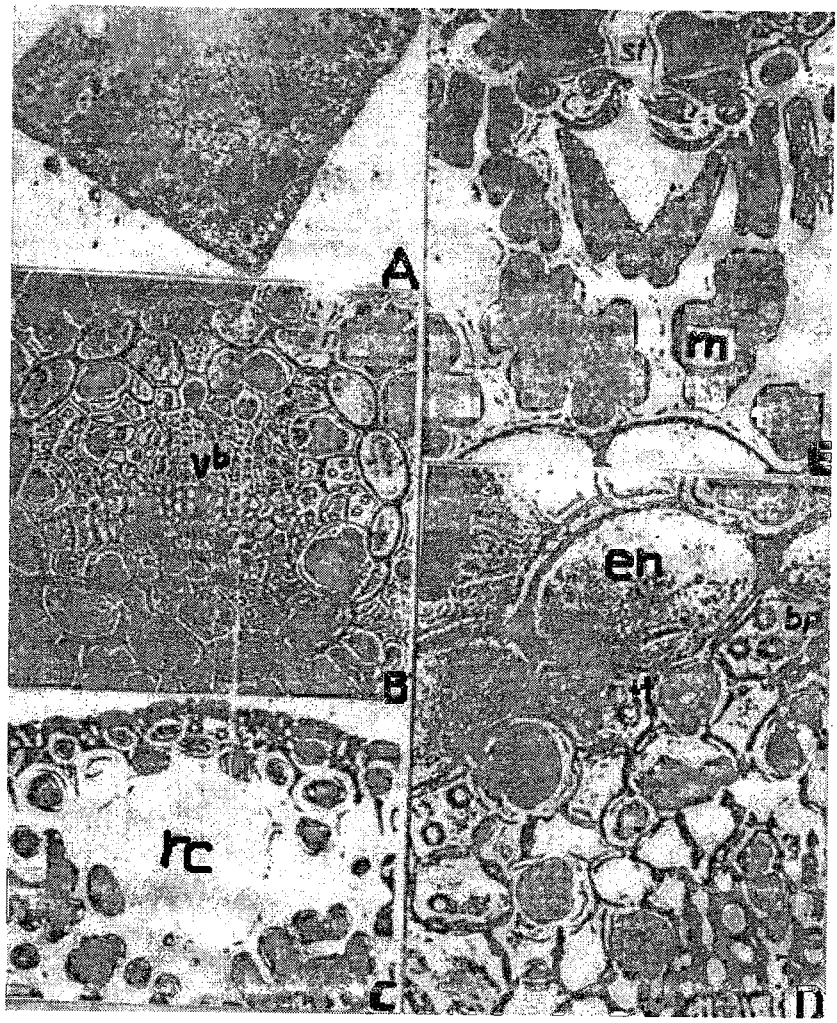


Plate 3

vb - Vascular bundle
rc - Resin canal
m - Mesophyll
bp - Border pits
en - Endormis

Plate 4

rc - Resin canal
bp - Border pits
m - Mesophyll

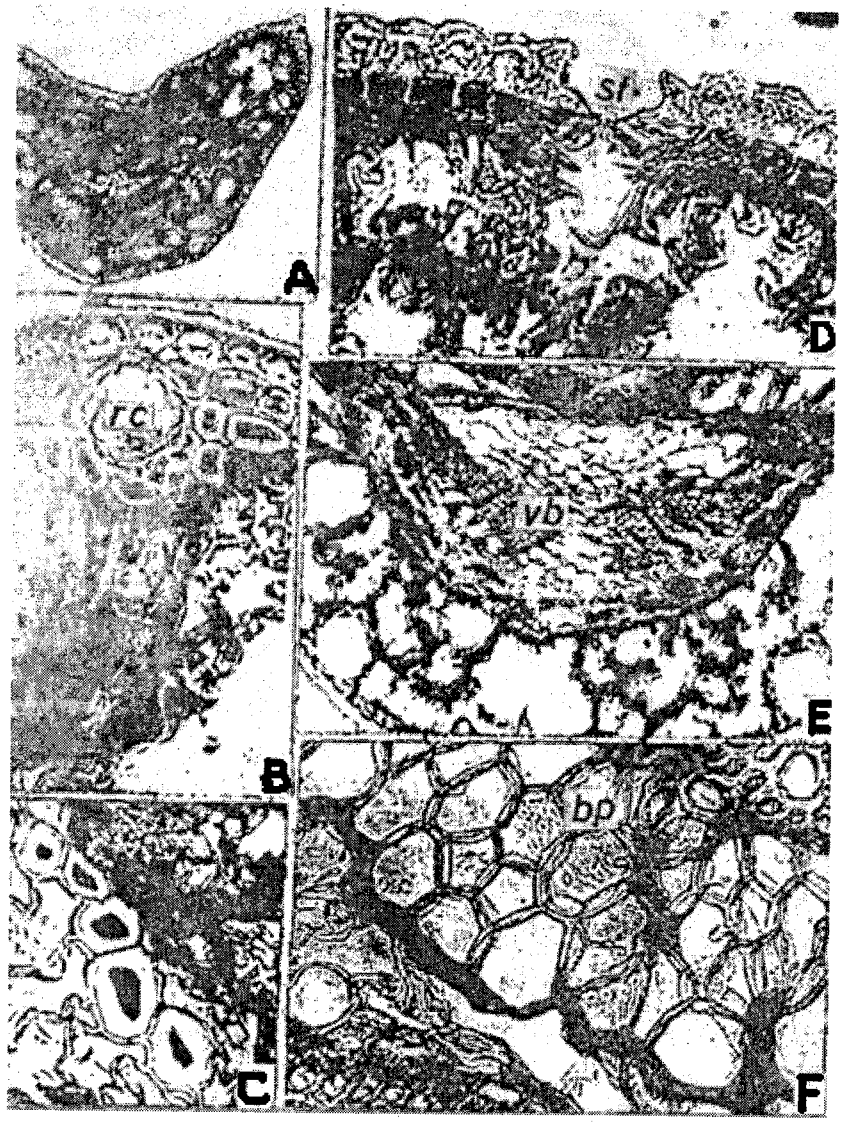


Plate 5

rc - Resin canal
st - Stomata
vb - Vascular bundle
bp - Border pits

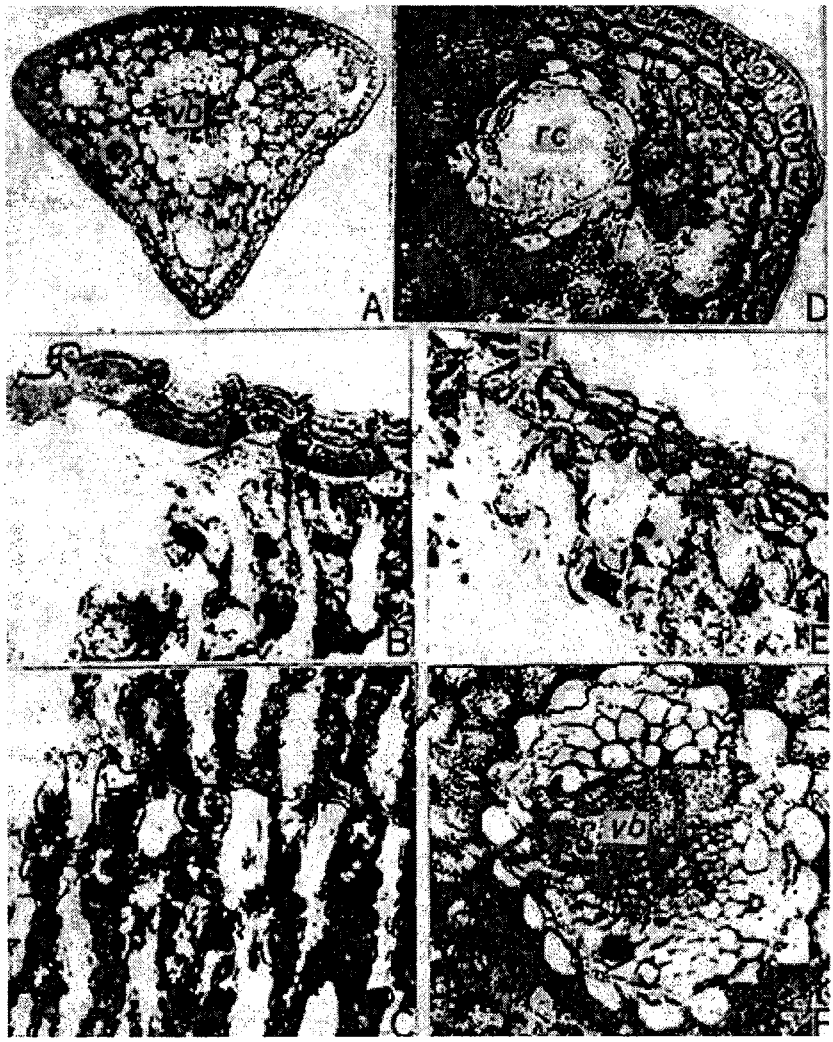


Plate 6

rc - Resin canal
st - Stomata
vb - Vascular bundle

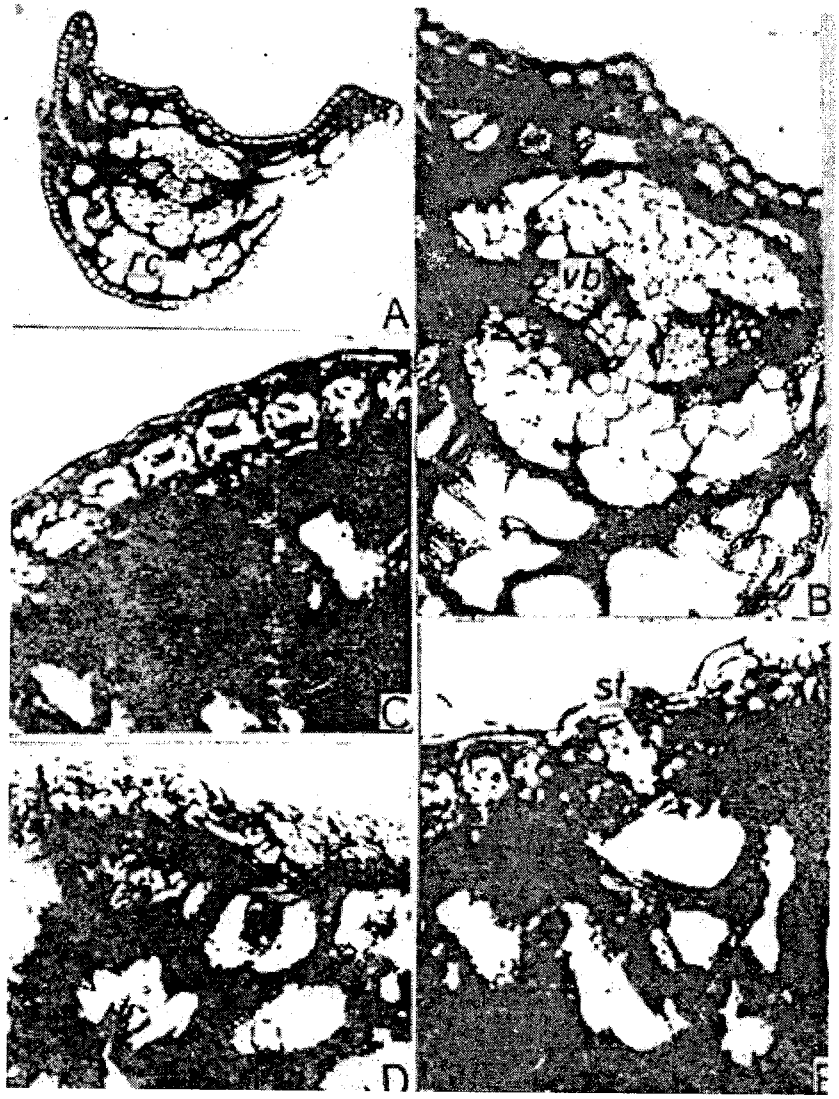


Plate 7

st - Stomata
vb - Vascular bundle

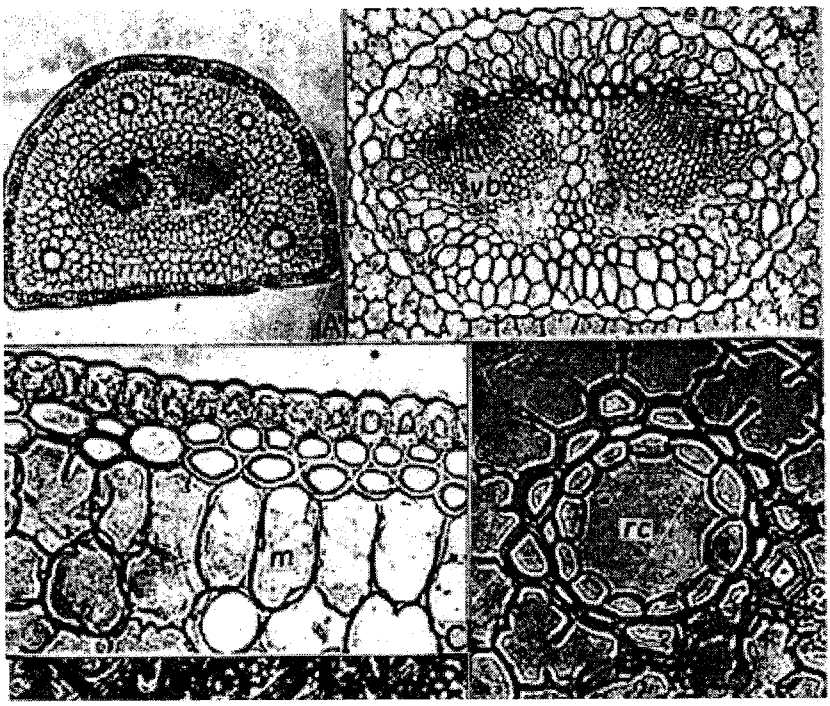


Plate 8

vb - Vascular bundle
m - Mesophyll
rc - Resin canal

Table 1. Epidermal features of *Pinus* needle leaves.

Taxa	Symmetry	Stomatal frequency st/mm^2	Stomatal index	Epidermal cells L x W (μm)	Polar subsidiary cells L x W (μm)	Guard cells L x W (μm)	Subsidiary cells L x W (μm)
P. densiflora	bifacial	86.0	89.5	160–140 x 100–80	62–42	30 x 16	4–6
P. echinata	trifacial	88.0	90.8	240–200 x 180–160	60–42	35 x 20	6–8
P. griffithii	trifacial	79.0	90.8	110–80 x 70–60	66–44	40 x 16	6–8
P. koriensis	bifacial	86.0	92.0	200–180 x 170–150	60–40	40 x 16	5–8
P. sylvestris	trifacial	94.2	96.0	100–60 x 40–80	62–40	33 x 23	4–8
P. tabulaeformis	trifacial	92.5	94.0	180–80 x 40–140	62–42	35 x 26	6–8

Table 2. Anatomical tissue of *Pinus* needle leaves.

Species	Shape in traverse section	Epidermal cells shape & size (μm)	Hypodermis	Mesophyll	Resin canal	Vascular bundle	Transfusion tissue
P. densiflora	Crescent shape	isodiametric 22 x 18	Single layered tangentially elongated cells	3–5 layered	4	2	Scanty
P. echinata	T shape	tangentially elongated 22 x 17.5	1 or 2 layered tangentially elongated cells	1–2 layered	3	1	Well developed
P. griffithii	T or wedge shape	tangentially elongated 16 x 14	1 or 2 layered tangentially elongated cells	1–2 layered	3	1	Well developed
P. koriensis	Triangular shape	tangentially elongated or nearly isodiametric 22.5 x 18	Single layer tangentially elongated	1–2 layered	3	1	Well developed
P. sylvestris	D shape	radially elongated 23.2 x 11.6	Single layered	3–5 layered	4–6	2	Well developed
P. tabulaeformis	Bowl shape	tangentially elongated	Single layered	2–3 layered	4–6	2	Well developed

SUMMARY, CONCLUSION AND RECOMMENDATION

Such type of study will be highly useful in identifying the various species of *Pinus* and hence it is of great taxonomic value. Besidès this it wil also be useful in identification of the fossil conifers.

The shape of needles in a transection has relation to the number of needles per dwarf shoot. It has been observed that in the species were the number of needles are 2 shape is semi-circular, D, or crescent shape but when the number of needles per dwarf shoot is 3 the shape is triangular. As described by Dolivo (1948) in other species of *Pinus*.

Florin (1931) in his fundamental work on morphology of conifers, studied the structure of epidermis and stomata in many conifers; he considered morphology of stomata to be useful for the study of evolution of conifers.

The number of resin canals indicate the resin yielding capacity of that particular species and hence we know which of the species of *Pinus* is more economically important and when growth will be more useful.

Shaw (1914) regarded the position of resin ducts to be of taxonomic value. The vascular bundle either single or double is surrounded with transfusion tissue that consists of thin walled, lignified tracheids and of living parenchyma cells as seen in my observations has been confirmed by Huber (1947).

Esua (1965) describes the occurence of two lateral resin canals as a constant feature in *Pinus* needles which has been confirmed by my observations which ranges from 2-6 Chaturvedi (1993, 1995). Further it has been confirmed that the number of needles in a given species tends to be constant and this forms a valuable taxonomic character.

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Antimicrobial Activity of Nine Common Plants in Kerala, India

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ABSTRACT

Nine common plants, namely, Aegle marmelos *(L.) Corr.,* Leucas indica *(L.) Vatka, Murraya konigii (L.) Spreng., Tamarindus indica (L.) Pachyptera alliacea (Lam.) A. Gentry, Eupatorium odoratum (L.), Moringa oleifera (Lamk.), Cinnamomum veerum (Preal) and Cymbopogon citratus (DC) Stapf. which are indigenously used in Kerala, India for curing various infections were tested against a fungus (*Aspergillus niger *) and Gram positive and negative bacteria (Staphylococcus aureus and Escherichia coli, respectively). Except for A. marmelos, all of the aqueous and alcoholic extracts of the plants at 5.0% concentration inhibited the growth of the microorganisms. The diameter (cm) of the inhibition zones of the 5.0% aqueous extracts ranged from 0.8 to 1.6 cm in A.* niger *and 1.0 to 1.4 cm in both S. aureus and E. coli. . Under the same concentration of alcoholic extracts, the inhibition zones have diameters ranging from 0.9 to 1.8, 1.0 to 1.8, and 1.0 to 2.0 cm for A. niger, S. aureus and E. coli , respectively. Alcoholic extracts of plants were consistently found to be more inhibitory than aqueous extracts of the same concentration. The alcoholic extracts (5.0%) of L. indica and C. citratus has the highest antifungal activity while E. odoratum has the greatest activity against both S. aureus and E. coli. .*

Key words: *Leucas indica, Cymbapogan citraus, Eupatorium odoratum,* antibacterial activity, antifungal activity, aqueous extract, alcoholic extract

INTRODUCTION

Research in antimicrobial activity of higher plants in India started seriously in the sixties and gained momentum in seventies. A large scale screening of Indian plants for biological activity was conducted by Dhar *et al.*, in 1968. Grainge and Alavarez in 1987 screened about 170 plants for their antimicrobial activity, and found that the leaf extract from *Artobotrys hexapetalus* was inhibitory. Antibacterial and antifungal activities of plant extract were carried out in many laboratories (Ferdous *et al* .,1992, Kodama *et al* .,1993, Amer *et al.*, 1994 and Purohit *et al.*, 1995). In all the previous studies, attention was given only to a particular family of plant and either to the alcoholic or aqueous extract. The study presents the results of antimicrobial screening of some medicinal plants in Kerala, India using both alcoholic and aqueous extracts from leaves alone or both from leaves and stem.

MATERIALS AND METHODS

Plants were mainly collected from Travancore and Malabar areas of Kerala. These were authenticated by Prof. V.V. Sivarajan (Dept. of Botany, University of Calicut, Kerala). Taxonomic listing of the plants are given in Table 1. Only the leaves and stem of the plants were used. The plant organs were thoroughly washed and dried in shade, ground and 20 g of the powder was successively extracted with double-distilled water and 95% ethyl alcohol in a Soxhlet extractor for 48 h. The aqueous extract was sterilized with Seitz filter. Both aqueous and alcoholic extracts were separately concentrated under reduced pressure. The residue left was weighed and dissolved in dimethyl sulfoxide (DMSO) to give a concentration of 5.0% (w/v). Diluted solutions of 2.5% (2-fold) and 1.25% (4-fold) were made from the original 5.0% solution.

Table 1. List of plants selected for the present study.

No.	Botanical name	Parts extracted
1	*Aegle marmelos* (L.) Corr	Leaves
2	*Leucas indica* (L.) Vatka	Leaves and Stems
3	*Murrya koenigii* (L.) Spreng	Leaves and Stems
4	*Tamarindus indica* (L.)	Leaves
5	*Pachyptera alliacea* (Lam.) A. Gentry	Leaves
6	*Eupatorium odoratum* (L.)	Leaves and Stems
7	*Moringa oleifera* (Lamk.)	Leaves and Stems
8	*Cinnamomum veerum* (Preal.)	Leaves
9	*Cymbapogon citratus* (DC) Stapf.	Leaves

The bacteria and fungus used in this study were obtained from the Department of Microbiology, Medical College, Calicut, Kerala. These DMSO extracts were screened for their antibacterial activity against *S. aureus* and *E. coli* and antifungal activity against *Aspergillus niger* by disc diffusion test (Maruzzella and Henry, 1958). Nutrient agar and potato dextrose agar were used to culture the bacteria and fungus, respectively . The bacteria were inoculated into nutrient agar broth and incubated at 37°C in a water bath and the suspensions were checked to approximately provide 10^5 cells/ml. From this 200 µl of suspension is transferred in the petri plates containing nutrient agar and the suspension was spread evenly on the medium with a glass spreader to get a uniform lawn of bacteria. For getting a fungal mat, spores of *A. niger* was suspended in 3 to 5 ml of normal saline solution taken in a test tube and the spore suspension was poured over a petri plates containing potato dextrose agar. Excess suspension was drained off. Whatman filter paper disc (No1, 0.5 cm diameter) was placed and test solution of the respective extracts in DMSO (50 µl) were aseptically added with the help of sterile syringes on separate paper discs. Streptomycin and griseofluvin were used as standards for comparison of antibacterial and antifungal activities respectively. These were added in the same manner. Then the plates were incubated at 25°C for fungus and at 37°C for bacteria.. Inhibition was recorded by measuring the diameter of inhibition zone at the end of 24h for bacteria and 72h for fungus. As control, the solvent (DMSO) in which extracts were dissolved was added on separate paper discs. Each experiment was triplicated and the average values are reported in the tables.

RESULTS AND DISCUSSION

All the plants showed antifungal activity at 5.0% of aqueous and alcoholic extracts (Table 2). The aqueous extracts of *M. koenigii, C. veerum* and *E. odoratum* showed antifungal activity even in 4- fold dilution . But the aqueous extracts of *M. oleifera, A. marmelos* and *P. alliacea* showed antifungal action only in 5.0% solution. *L. indica, T. indica* and *C. citratus* did not show any action in their 4- fold dilution (Table 2). Alcoholic extracts of all the plants except *A. marmelos* and *L. indica* showed antifungal activity even in 4- fold dilution (Table 2). Most striking antifungal action was shown by *L. indica, C. citratus* and *E. odoratum* . Their antifungal action in 5.0% solution of both aqueous and alcoholic extract was almost same (Table 2). The antifungal action of *C. citratus* , *C. veerum* and *M. koenigii* may be due to their oils and its derivative (Lima *et al* ., 1993, Pandey and Dubey 1994).

Table 2. Diameter (cm) of inhibition zones of the different concentration of alcoholic and aqueous extracts of nine plants on the microorganisms.

Plant name	Con. (%)	Microorganisms					
		A. niger		*S. aureus*		*E. coli*	
		Alcoholic	Aqueous	Alcoholic	Aqueous	Alcoholic	Aqueous
A. marmelos	5.00	0.9	0.9	1.00	1.0	1.00	0.0
	2.50	0.0	0.0	0.00	0.0	0.00	0.0
	1.25	0.0	0.0	0.00	0.0	0.00	0.0
L. indica	5.00	1.8	1.6	1.0	1.4	1.6	1.2
	2.50	1.5	1.0	0.8	0.0	1.4	1.1
	1.25	1.0	0.0	0.0	0.0	1.2	1.0
M. koenigii	5.00	1.4	1.2	1.5	1.0	1.4	1.0
	2.50	1.2	0.8	1.2	0.0	1.1	0.9
	1.25	1.0	0.5	0.8	0.0	0.8	0.0
T. indica	5.00	1.5	1.4	1.2	1.0	1.5	1.0
	2.50	1.0	1.0	1.0	0.0	1.2	0.0
	1.25	0.8	0.0	0.0	0.0	1.0	0.0
P. alliacea	5.00	1.4	1.1	1.0	1.0	1.2	1.1
	2.50	1.0	0.0	0.9	0.0	1.1	0.9
	1.25	0.8	0.0	0.0	0.0	1.0	0.8
C. veerum	5.00	1.2	1.0	1.2	1.0	1.5	1.0
	2.50	1.0	0.8	1.0	0.9	1.2	0.9
	1.25	0.8	0.6	0.8	0.0	1.0	0.0
C. citratus	5.00	1.8	1.6	1.3	1.0	1.6	1.0
	2.50	1.6	0.6	1.0	0.8	1.0	0.9
	1.25	1.4	0.0	0.8	0.0	0.9	0.8
E. odoratum	5.00	1.6	1.5	1.8	1.2	2.0	1.4
	2.50	1.2	0.7	1.5	1.0	1.6	1.1
	1.25	0.8	0.5	1.2	0.8	1.4	1.0
M. oleifera	5.00	1.0	0.8	1.4	1.0	1.4	1.0
	2.50	0.0	0.0	1.2	0.0	1.1	0.0
	1.25	0.0	0.0	1.0	0.0	1.0	0.0

A more detailed perusal of results (Table 3) showed that using alcoholic extracts, all the nine plants studied exhibited antifungal activity at 5.0% concentration of the extracts. At 2-fold and 4-fold only two plants namely *A. marmelos* and *M. oleifera* failed to respond. Antibacterial activity against *S. aureus* and *E. coli* was also shown by all the plants at 5.0% concentration. At 2-fold dilution 8 plants retained inhibitory action against both organism. However at 4-fold dilution of extracts only 5 plants were effective against *S. aureus* in comparison to 8 plants with *E. coli.*

Table 3. Number of plants showing inhibition to the microorganisms and the range of diameter (cm) of inhibition zones at different concentrations of alcoholic extracts.

Microorganisms	Concentrations	No. of Plants Total No = 9	Diameter (cm) of inhibition zones
A. niger	5.00	9	0.9 – 1.8
	2.50	7	1.0 – 1.6
	1.25	7	1.0 – 1.6
S. aureus	5.00	9	1.0 – 1.8
	2.50	8	0.8 – 1.5
	1.25	5	0.8 – 1.2
E. coli	5.00	9	1.0 – 2.0
	2.50	8	1.0 – 1.1
	1.25	8	0.8 – 1.4

The responses given by aqueous extracts are given in Table 4. Though all the nine plants inhibit *A. niger* growth at 5.0% level, at 2-fold and 4-fold dilution's only 6 and 3 plants respectively were effective. Against *E. coli*, the responses at 5.0%, 2.5% and 1.25% concentration were exhibited by 8,6 and 4 plants respectively. The most prominent observation was the lack of inhibition of *S. aureus* growth by aqueous extract at lower concentrations. All nine plants showed inhibition of growth at 5.0% and at 2-fold dilution, only 3 showed activity and at 4-fold dilution only one plant was effective.

Table 4. Number of plants showing inhibition to the microorganisms and the range of diameter (cm) of inhibition zones at different concentrations of aqueous extracts.

Microorganisms	Concentrations	No. of Plants Total No = 9	Diameter (cm) of inhibition zones
A. niger	5.00	9	0.8 – 1.6
	2.50	6	0.6 – 1.0
	1.25	3	0.5 – 0.6
S. aureus	5.00	9	1.0 – 1.4
	2.50	3	0.8 – 1.0
	1.25	1	0.8
E. coli	5.00	8	1.0 – 1.4
	2.50	6	0.9 – 1.1
	1.25	4	0.8 – 1.0

The zones of inhibition obtained from 5.0% solution of aqueous and alcoholic extracts of *E. odoratum* is 1.2 cm and 2.0 cm respectively . This corresponds to zone of inhibition obtained for 100 mg of Streptomycin used as standard. Both aqueous and alcoholic extracts of *E. odoratum* showed antibacterial activity against Gram negative bacteria. *Chromolaena odoratum* which belongs to the same family (Copositae) also showed antibacterial action (Irobi, 1992). Four fold dilution of aqueous extract of *P. alliacea* and *C . citratus* showed antibacterial activity against Gram negative bacteria while the 4- fold dilution of *C. veerum* , *M. koenigii* and *M. oleifera* did not have any effect against Gram negative bacteria. The antimicrobial action of *P. alliacea* was also reported by Sharma (1993). The greatest antibacterial activity against

Gram negative bacteria was shown by *E. odoratum* . The antibacterial action of *M. oleifera* and *A. indica* was reported by Mendia *et al* (1991) and Iyer and Williamson (1991).

The study shows that the alcoholic extract has a better antifungal action than aqueous extract. This is in agreement with the report of Islam *et al* (1992). That the antibacterial/antifungal activity of the alcohol extractable fraction was due to more than one active principles was confirmed following TLC separation of components (data not shown). This finding merits further detailed studies as regards the chemical nature of the compounds particularly *L. indica* , *C. citratus* and *E. odoratum* for promising antifungal agents. The use of E. *odoratum* and L. *indica* in Kerala for various skin infections is justified by this work, as it showed commendable activity against both the test organisms.

SUMMARY

L. indica, *C. citratus* and *E. odoratum* has high potentials as an antifungal and antibacterial agents, respectively. The alcohol extractable fraction elicited greater activities than the aqueous extracts from the plants. The presence of one or more active principles is also indicated. Identification of the active principles from these plants must be carried out as they may be new and novel sources of both antifungal and antibacterial compounds.

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A Correlation Study of Pupal Size to Egg Fecundity and Silk Yield of Silkworm (Bombyx mori Linn.)

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ABSTRACT

A comprehensive study on the correlation of pupal size to egg fecundity and silk yield of silkworm, Bombyx mori was made. The results revealed that most of the eggs were mature at the time of oviposition. The number of mature eggs was highly correlated with total number of eggs ($r = 0.99$). All the pupal dimensions, namely body weight, body length, body width, and wing length could be considered equally important parameters for correlation with total number of eggs and number of mature eggs. However, pupal weight was found to be the best one for estimating both number of mature eggs ($r = 0.63$) and total number of eggs ($r = 0.62$). Correlations of pupal weight were positive with shell weight ($P < 0.001$), cocoon weight ($P < 0.001$), filament length ($p < 0.05$) and total number of eggs ($p < 0.05$). This indicates improvement in silk yield attributes and in the total number of eggs during pedigree selection in silkworm breeding programmes.

KEY WORDS: Pupal dimensions, Egg production, Silk yield attributes, Correlations.

INTRODUCTION

As many as 21 characters of the silkworm, *Bombyx mori*, contribute to silk yield (Thiagarajan *et al.*, 1993). In fact, silkworm breeding depends, to a large extent, on the mutual relationships between these characters (Safonova & Dehkanov, 1976; Fengling, 1989; Nacheva, 1989; Li, 1992). Hence, for planned breeding programmes to improve the silk yield, information on the association of these characters with cocoon yield and among themselves is necessary (Pan *et al.*, 1995).

Correlation measures the degree of association between two characters. Information on correlation between the morphological characters of the silk moth, like pupal dimensions and egg number, and technological characters, like cocoon weight, shell weight, raw silk percentage, filament length, reelability, and neatness, are of immense importance in the pedigree selection programme.

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The larger and heavier female pupae are selected for production of egg layings with greater number of eggs. The relationship between certain dimensions of female pupae and fecundity have been described by a few workers in the recent past. In fact, a significant relationship between wing length and number of mature eggs produced by the female of *Crambus harpipterus* and *Agriphila plumbifimbrella* was shown by Crawford (1971), while a similar relationship between either wing length observed in *Rhyacionia neomexicana* by Jennings (1974). In addition, the relationship between pupal size and egg production in females of *Antheraea polyphemus* was studied by Miller *et al.* (1982). Such information would be valuable during pedigree selection of individuals from a population in silkworm breeding programmes. Hence, in females of *B. mori*, an attempt has been made to determine the phenotypic correlations between pupal dimensions and egg production on the one hand, and the post cocoon reeling parameters on the other.

MATERIALS AND METHODS

Healthy cocoons of CC1 sex-limited cocoon colour race of *B. mori* (n = 450) were taken for the present study. A sex-limited cocoon colour race is one in which all yellow colour cocoons are females and all white ones are males (Nagaraiu, 1996). On the 5th day of cocooning, the female pupas (n = 150) were taken out randomly from the cocoons and the freshly formed pupae were weighed and measured. Average cocoon weight (g) included both pupal weight (g) and shell weight (g). The remaining 300 cocoons were processed for reeling according to Bhargava (1995). The average values for 7 silk yield attributes, namely cocoon weight (g), shell weight (g), pupation rate (%), raw silk (%), filament length (m), reelability (%), and neatness (points) were obtained from the reeling results of cocoons as described by Bhargava *et al.* (1995).

Pupal body weight (Wt), body length (Bl = measured as distance from vertex of head to posterior end of the abdomen, body width (BW = width of fourth abdominal segment) and wing length (WI = distance from the vertex to the apex of left wing pad) were taken into consideration. After oviposition, the adult females were dissected and the unoviposited mature and immature eggs were counted. Maturity of eggs was determined by staining with Grenacher's Borax Carmine (Humason, 1972), immature eggs stained red while the mature eggs did not stain. The eggs were treated (within 24 h of oviposition) with hydrochloric acid of 1.075 specific gravity and 46.1°C temperature, to break the diapause for hatching. Egg layings were incubated under optimum environmental conditions following the standard method. The hatched, unhatched, and unfertilized eggs were counted, and hatching percentage was calculated. Data were subjected to statistical analysis for identification of important pupal characters related with egg production.

RESULTS AND DISCUSSION

The different pupal measurements i.e. pupal weight (Wt), body length (Bl), body width (Bw), wing length (Wl) and their combinations were observed to determine their relationships with the number of mature eggs and total eggs. Results are presented in Tables 1 and 2.

Table 1. Summary of pupal measurements and number of eggs observed in female *Bombyx mori*.

Measurements	Mean+SD
Wt (g)	1.313 ± 0.15
Bl (mm)	25.30 ± 1.36
Bw (mm)	9.40 ± 0.52
Wl (mm)	10.82 ± 0.69
NME	543.53 ± 66.26
TE	546.20 ± 65.75
Hathced eggs (%)	94.45 ± 3.85

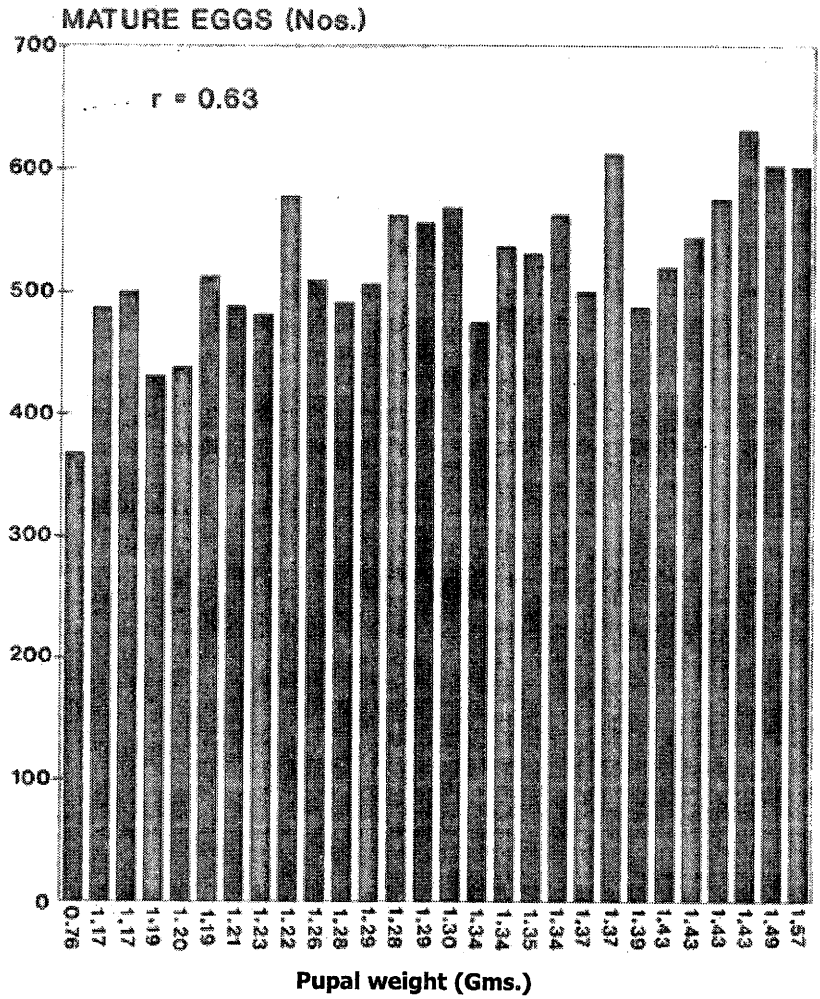
Wt, pupal weight; Bl, body length; Bw, body weight; Wl, Wing length; NME, number of mature eggs, and TE, Total eggs

Table 2. Relationship between pupal measurements and number of eggs observed in female *Bombyx mori*.

Pupal Measurements	Correlation (4) with no. of	
	Mature eggs	Total eggs
Wt	0.63**	0.62**
Bl	0.43*	0.42*
Bw	0.37*	0.36*
Wl	$0.28^{N.S}$	$0.28^{N.S}$
Wt x Bl	0.59**	0.58**
Wt x Bw	0.62**	0.60**
Wt x Bl	0.59**	0.58**
Bl x Bw	0.53**	0.51**
Bl x Wl	0.42**	0.43**
Bw x Wl	0.42**	0.41**
Wt x Bl x Bw	0.60**	0.59**
Wt x Bl x Wl	0.57**	0.57**
Wt x Bw x Wl	0.59**	0.58**
Bl x Bw x Wl	0.51**	0.50**
Wt x Bl x Bw x Wl	0.59**	0.58**

*** and **: significant at 5% and 1% level, respectively.**
N. S. = non-significant

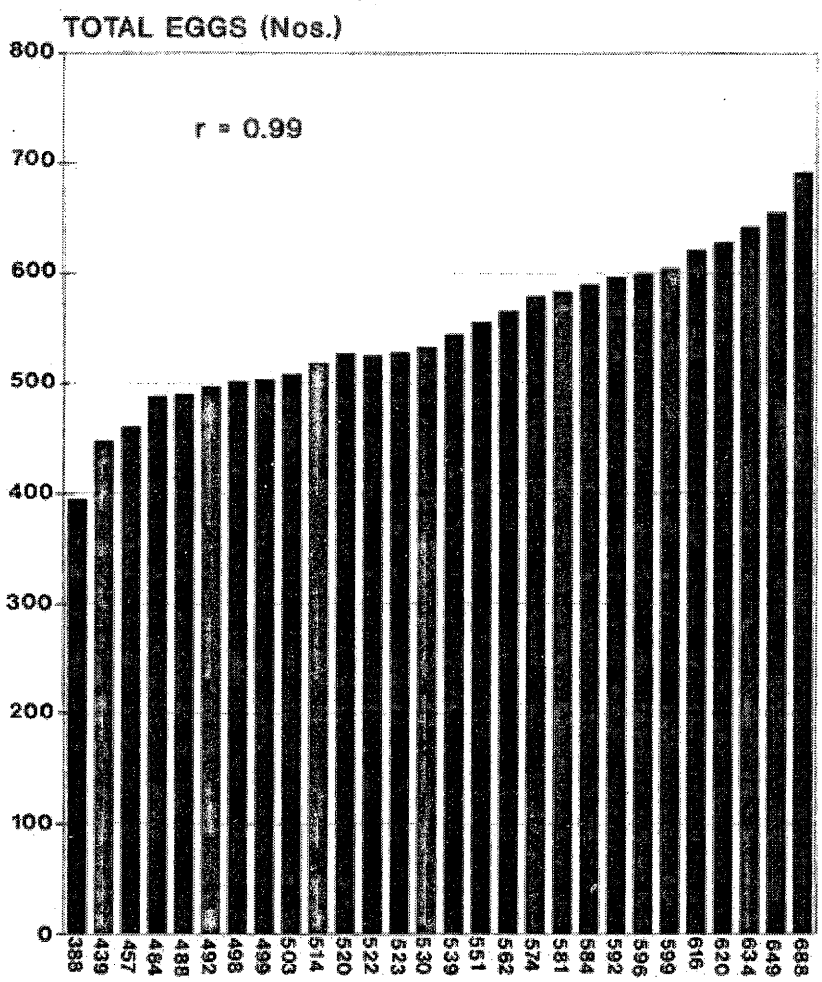
Figure 1. Number of mature eggs in Bombyx mori female as a functions of pupal weight.



After oviposition, most of the eggs were found mature in *B. mori* (543.53+66.26). Correlation coefficients (r) between various pupal dimensions and number of mature eggs or total eggs varied from 0.28 to 0.63 (Fig.1, Table 2). The highest correlation was estimated between the pupal weight (Wt) and number of mature eggs (r = 0.63) and total eggs (r = 0.62). Similar observations were reported in another lepidopteran, *Antheraea Polyphemus* (Miller *et al.*, 1982). In the present study, a negative and non-significant correlation (r = 0.28) was observed for both mature eggs and total eggs with wing length (Table 2). However, a significant correlation between wing length and number of eggs was observed in *Grambus harpipterus* (Crawford, 1971), *Rhyacionia neomaxicana* (Jennings, 1974) and in *Antheraea Polyphemus* (Miller et

al., 1982). There was a positive and highly significant correlation (r = 0.99) between number of mature eggs (NME) and total eggs (TE) (Fig. 2). These results support the earlier investigation on *Antheraea Polyphemus* (Miller *et al.* 1982). A mean pupal weight (Wt) of 1.313 g produced 546 eggs (Table 1). Hence, 416 eggs can be obtained from 1 g of pupal weight of *B. mori.*

Figure 2. Relationship between number of mature eggs and total eggs in female Bombyx mori.



The results on phenotypic correlation coefficients between nine silk yield attributes presented in Table 3 are in agreement with earlier reports on *B. mori* (Nacheva, 1989; Tzenov et al., 1994). It is noted that pupal weight showed positive correlations with shell weight ($p < 0.001$), cocoon weight ($p < 0.001$), filament length ($p < 0.05$) and total number of eggs ($p < 0.05$).

Table 3. Estimates of phenotypic correlation coefficients for silk attributes in *Bombyx mori*.

Character	Shell weight	Cocoon weight	Pupation rate	Raw silk %	Filament length	Reelability	Neatness	Total number of eggs
Pupal weight	0.72***	0.84***	–0.12	0.05	0.26*	–0.05	0.12	0.29*
Shell weight		0.61*	–0.19	0.28*	0.59**	–0.09	0.04	0.27*
Cocoon weight			–0.09	0.07	0.44**	–0.10	0.10	0.28*
Pupation rate				0.35**	0.17*	0.01	0.06	0.04
Raw silk %					0.39**	0.38**	0.16	0.04
Filament lenght						0.26*	0.31**	0.01
Reelability							0.10	–0.13
Neatness								0.08

*** $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$**

This indicates that in silkworm breeding programmes aimed at a greater number of eggs per laying, the choice for high pupal weight during pedigree selection may also result in high shell weight and cocoon weight along with longer filament length in the offspring of individuals subjected for selection.

In conclusion, though body weight, body length, and body width were significantly correlated to the number of mature eggs and total number of eggs, pupal weight was found to be the best parameter for estimating them in *B.mori*. Hence, pupal weight may also help to some extent in estimating the production of the exact quantity of eggs by number, right at the time of preparing layings in granuers. Furthermore, the impact of pupal weight on the silk yield attributes as seen from the positive and negative correlation coefficients in the present study, offers the breeder very useful information for improving silk quality.

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